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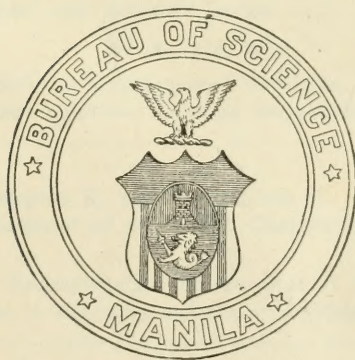
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THE PHILIPPINE  
JOURNAL OF SCIENCE

B. TROPICAL MEDICINE

VOL. XIII

JANUARY, 1918

No. 1

SOME OBSERVATIONS AND EXPERIMENTS ON MALAYAN ANOPHELES WITH SPECIAL REFERENCE TO THE TRANSMISSION OF MALARIA<sup>1</sup>

I, EXPERIMENTAL AND NATURAL INFECTION OF INSECTS WITH MALARIA, WITH SOME NOTES ON THE MORPHOLOGY AND BIOLOGY OF CERTAIN TYPES OF ANOPHELES ROSSI

By MARSHALL A. BARBER<sup>2</sup>

(*Kuala Lumpur, Federated Malay States*)

TWO TEXT FIGURES

The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Board of the Rockefeller Foundation.

The work was done for the most part during 1915 and 1916, and most of the anophelines studied were collected in Selangor State, Federated Malay States. Certain details regarding the method of feeding mosquitoes on gamete carriers, selection of material, dissection, and the like will be given in connection with the tables or included in the description of the technic.

In the identification of Malayan *Anopheles* I have depended chiefly on the publications of Stanton, and I owe much to him for personal aid in identifying certain specimens. The works of Watson, Strickland, Leicester, and other observers in Malaya have also been of much assistance, not only in the classification, but also in the study of biological and epidemiological characteristics of species.

<sup>1</sup> Received for publication September, 1917.

<sup>2</sup> Bacteriologist in the Bureau of Science, Manila, July, 1911, to April, 1915.—EDITOR.

A good deal of work was done on varieties of *A. rossi*. The large number and the wide distribution of these forms make them of interest from the epidemiological point of view, and the abundance and accessibility of material made them a favorable subject for the study of certain problems in infectivity with malarial parasites.

At the time when these experiments were begun, but one variety of *A. rossi* had been reported from the Malay Peninsula, namely, *A. rossi* var. *indefinitus* Ludl. In the earlier feeding experiments most of the material was collected in brackish water near Port Swettenham. Here the imagoes varied little, and it was assumed, probably on good grounds, that all were var. *indefinitus*. Later a quantity of material was collected from fresh water, in particular from a certain group of fish ponds near Kuala Lumpur. In this lot of material much variability was observed in the breadth of the first black palpal band. There was a continuous series of specimens varying from those exhibiting no such band to those with a terminal black almost equal in length to the terminal white. Some specimens with the broader band together with the corresponding larval skin were submitted to Doctor Stanton, who noted that larva and imago were similar to specimens collected by him in Java. He subsequently sent some of the Java specimens to the British Museum, where they were identified as *A. rossi* Giles, on the basis of the larval characters and of the broader black band. Dr. S. T. Darling had previously noted wide variation in the larvæ of *A. rossi* collected near Kuala Lumpur. It seemed probable, then, that we had to do with two types of *A. rossi*, namely, *A. rossi* var. *indefinitus* and *A. rossi* Giles or a form somewhat similar to it, and further studies were undertaken regarding the characteristics of these two types. For the sake of brevity in description the two Malayan types will be referred to as "type *indefinitus*" and "type Giles."

In the course of comparative infection experiments carried out with these two types and in connection with other observations, over 6,500 larvæ and many hundred adults were examined. Some of the results of these observations are summarized as follows:

*Larva*.—The chief characteristics of the larva of the two types correspond to the published descriptions of *A. rossi* var. *indefinitus* and of *A. rossi* Giles, respectively. The principal dif-



ference between the two forms in the larva lies in the character and arrangement of the clypeal hairs. In *indefinitus* the anterior internal pair are long, the anterior external relatively short, and the posterior pair very short and situated near the interior pair, not directly behind these but in such a position that lines drawn in a direction directly anterior to the posterior pair would divide the space between the anterior internal into three approximately equal parts. In type Giles the external anterior pair are somewhat longer, and the posterior pair, which are somewhat longer than those of var. *indefinitus*, lie much farther back and are nearly directly behind the anterior internal pair. Some variations in size and position occur in these types, but the combination of characters is such that the observer is rarely left in doubt as to which type a given larva belongs.

While the clypeal hairs of both types are typically simple, I noted various adventitious forkings and branchings, and these were by far more frequent in type Giles. These branchings varied from a simple forking of one of the anterior internal hairs to a branching or forking of four or more hairs and sometimes to a duplication of hairs. The first variation mentioned was by far the most frequent. The branching was noted in mature larvæ, but was more frequent in young larvæ. Some small larvæ with these adventitious branchings were isolated and examined two days later, when they were found to have lost the branches. Larvæ were examined under a cover glass, and where there was any doubt of the presence of these anomalies, specimens were examined under higher powers of the microscope, in order to exclude possible error from the confusion of branching with the appearance afforded by the crossing of hairs.

In 3,019 larvæ of type Giles anomalies of clypeal hairs in some degree were noted in 130, or 4.3 per cent. In 3,525 larvæ of type *indefinitus* they were noted in only 2, less than 0.1 per cent. Possibly many anomalies were overlooked, since the matter was more or less incidental to other observations, but in comparing the two types, both examined in the same way and both of various ages, it is evident that type Giles is more variable in this respect. The matter may be of small weight in itself, but taken in connection with the greater variability of adult type Giles, it goes to show that type Giles is the less stable form. Further we are put on our guard against attaching much specific or varietal value to minor variations in clypeal hairs.

*Adult.*—The only adult character that appears to be of any value in distinguishing females of the two types is the length of the first black palpal band. The variability of this character is shown in Table I, where females are compared according to the ratio that the length of the first black band bears to the length of the portion of the palp covered by this band and the terminal white band, or

$$\frac{\text{first black}}{\text{first black} + \text{terminal white}}$$

All specimens included in these tables had been previously identified in the larval stage.

In the smaller group under A, of Table I, palp measurements were made under the low power of a compound microscope by means of an eyepiece micrometer. The insects were chloroformed and, while still fresh, placed each on a slide in a definite position; the palps were then covered by a small cover glass in such a way as to bring them parallel to the slide. The same specimens were measured by means of a hand lens without a scale. The hand lens measurements are entered under B, of Table I, for comparison. The line of demarkation between the bands, usually definite under the hand lens, sometimes appeared irregular under the high power. Further, terminal scales that do not get their full value under the lens may be included in the high-power measurement. The two sorts of measurement, therefore, cannot be expected to agree exactly. However, the aim was simply to compare approximately the variability of the two types in regard to a certain character, and in that respect the hand-lens measurement appears to agree closely enough with the more accurate method to suffice. In C larger numbers are included and only hand-lens measurements are given. Since many of the anophelines included under C were to be used subsequently for infection experiments, it is obvious that they could be examined only in the living stage. They were measured in test tubes, usually but one or two in a tube, and were viewed as nearly as possible at right angles to the palps. The hand-lens measurements are only approximate, but as stated above, the approximate agreement of measurements under A and B of the table indicate that the hand-lens measurements were sufficiently accurate to show, in a general way, the amount and character of the variability in length of the terminal palpal bands of the two forms.



TABLE I.—*Palp ratios of different varieties of A. rossi. Incidence of cases.*

## A. EYEPIECE MICROMETER MEASUREMENT.

Ratio.	Type Giles, brackish water.	Type Giles, fresh water.	Var. in- definitus, fresh water.	Total.	Ratio.	Type Giles, brackish water.	Type Giles, fresh water.	Var. in- definitus, fresh water.	Total.
0.00			2	2	0.25	2	4	1	7
0.01			1	1	0.26	2	5	1	8
0.10			2	2	0.27	3	5		9
0.11			2	2	0.28	3	1	1	5
0.12			1	1	0.29	6	4	1	11
0.13		1		1	0.30	10	1		11
0.14		1	2	3	0.31	4	4		8
0.15			3	3	0.32	3	4		7
0.16		1	2	3	0.33	7	4		11
0.17			4	4	0.34	7			7
0.18		1	7	8	0.35	5			5
0.19		1	4	5	0.36	5			5
0.20			5	6	0.37	1	2		3
0.21			5	5	0.38	4			4
0.22	2	2	5	9	0.40	3	1		4
0.23	1	2	1	4	Total	70	49	53	172
0.24	2	4	2	8					

## B AND C. HAND-LENS MEASUREMENT.

Ratio.	B. Same specimens as A.				C. All specimens, including A and B.		
	Type Giles, brackish water.	Type Giles, fresh water.	Var. in- definitus, fresh water.	Total.	Type Giles, brackish and fresh water.	Var. in- definitus, fresh water.	Total.
0.0			2	2		15	15
0.1—					1	12	13
0.1			2	2	2	37	39
0.1+			2	2	7	47	54
0.2—		1	5	5	14	120	134
0.2		2	15	17	64	209	273
0.2+	4	8	21	33	191	61	252
0.3—	23	13	4	40	118	9	127
0.3	17	12		29	109	4	113
0.3+	19	7		26	97	1	98
0.4—	7	6		13	64		64
0.4					48		48
0.4+					25		25
0.5—					3		3
0.5					13		13
Total	70	49	51	170	756	515	1,271

It will be observed in Table I that type *indefinitus* has the narrower range and, in general, the less variability. The amount of overlapping of the two types is such as to indicate the impossibility of differentiating female adults of these types by palpal ratios alone, unless the first black band should have a length near the maximum of type Giles or the minimum of type *indefinitus*. No other adult character has been thus far noted sufficiently constant to differentiate these forms. Type Giles of brackish-water origin seems to have less variability than the fresh-water forms. However, all of the brackish-water type Giles were collected in one locality, and collections from a variety of brackish-water habitats might show a greater amount of variability. To sum up, the data given in this table indicate that it is difficult, if not impossible, to describe adults of certain closely related members of the *rossi* group without recourse to the statistical study of many specimens. Larval characteristics must be taken into account in differentiation, and possibly breeding experiments will be necessary to furnish the final data for the classification of some more closely related types.

*Habitat.*—The larvæ of type *indefinitus* of Malaya are most commonly found in muddy pools exposed to the sun, and the greater part of my material was collected in such places. *Anopheles rossi* Giles of India is reported to frequent similar places. In Malaya, however, so far as my observation has gone, larvæ of type Giles are never found in such pools, but frequent ponds or large pools relatively clear and supplies with grass or other vegetation. They are commonly found associated with *fuliginosus*, *barbirostris*, and *sinensis*. In the collection of many hundreds of larvæ in small muddy pools, I have never once found this type. Type *indefinitus*, while commonest in muddy pools, seems to be less selective in its habitat and is found in a variety of places. It is sometimes associated with type Giles in ponds. On a number of occasions I have found small muddy pools containing only type *indefinitus*, while a few feet away ponds contained type Giles in abundance.

The relatively restricted habitat of type Giles, especially its absence from small muddy pools, would be evidence that it is phylogenically distinct from type *indefinitus* and would raise the question as to whether it may not differ, biologically at least, from *A. rossi* Giles of India.

No microscopical character was noted that was of much use in distinguishing the two types of Malaya in collecting. Type *indefinitus* of muddy pools seems slightly larger than type Giles,



but when both are found together in ponds they appear indistinguishable to the naked eye. When occurring in ponds, in the younger stage both much resemble *A. fuliginosus* macroscopically.

Type Giles appears to be rather common in the Federated Malay States. It was found by me in seven different places within 6 or 7 kilometers of Kuala Lumpur. In one of these places—certain fish ponds—they could be collected in large numbers throughout a period of several months, and they were abundant in two other ponds. I also found them in fresh water at Tronoh, Perak. In brackish water many were collected in some pools well supplied with algæ at Port Weld, Perak. The brackish-water larvæ seemed a little darker in color and the adult slightly darker than in the fresh-water type, though probably this was only a slight local variation. The occurrence of this type in brackish water, the ordinary breeding place of *A. ludlowi* in Malaya, is noteworthy, since the larva of type Giles and that of *ludlowi* appear identical. It is evident that adult as well as larval stages are necessary in distinguishing these forms.

#### SUSCEPTIBILITY TO PARASITES OF MALARIA

In comparing the two types of *rossi* in regard to susceptibility to malaria, five pairs of caged anophelines were fed on gamete carriers, one member of a pair type Giles, the other, type *indefinitus*. All specimens were examined in the larval stage and the types separated before emergence. Each pair of cages was fed at the same time and on the same gamete carriers. In two of the pairs both types were collected from the same pond and at the same time. The members of a pair are almost exactly comparable, except that in two pairs some mosquitoes that emerged late were introduced into cages after the cages had been once exposed to a carrier. The majority of those introduced later were of type Giles, so that any error from this source, if such exists, would tend to reduce the percentage infected of type Giles. In every pair but one type Giles showed at dissection much the higher percentage of infected mosquitoes. In the exception 3 out of 4 *indefinitus* dissected were infected and none out of 3 type Giles. The results of this comparison are summarized in a small table appended to the bottom of Table II. It will be seen that of type Giles 37.2 per cent were infected of 94 dissected, while of type *indefinitus* only 11.0 per cent were infected of 73 dissected. Type Giles gave on the average twice

as many zygotes per infected mid-gut and a larger percentage of sporozoites than type *indefinitus*. The only sporozoites found in *indefinitus* occurred in the mid-gut and were apparently degenerate.

A curious difference was observed in the tendency to development of ova in the two types. In 3 out of 5 cages and in 39.5 per cent of the total number dissected type Giles showed ova well advanced in development, often as early as the sixth day after feeding, while well-developed ova never appeared in type *indefinitus*. In 4, at least, of the 5 pairs males were included with the females in cages.

Some further observations on these two types and on other types of *rossi* will be made in connection with certain tables and in the summary at the close of this paper. From the comparative observations on the two types there seems to be substantial evidence for regarding them as distinct forms, biologically and morphologically as well, even though they may not be distinguishable in the adult stage. Further the question may be raised whether type Giles of Malaya may not be a different form from *A. rossi* Giles of India. In view of the great variability of the Malayan type, its restricted breeding places, and its susceptibility to infection with malarial parasites, it may be at least a biological variant, its characteristics dependent in some measure on its topographical environment.

In Table II are summarized the laboratory experiments on infection with malarial parasites of various species of Malayan *Anopheles*. Only controlled lots are included in this table, that is, lots in which at least one insect of some species became infected at a feeding. In other words, one or more of the gamete carriers used had viable gametes at the time the feeding was done. In regard to the species included, *A. ludlowi* is the common brackish-water species of Malaya, having a larva like that of type Giles and an adult resembling *A. rossi*, but with distinctly speckled legs. In the specimens I have observed the length of the first black palpal band is similar to that of the broader banded specimens of type Giles as described above, but apparently *ludlowi* varies less than type Giles in this respect. *Anopheles ludlowi* of Malaya seems to be usually a brackish-water type, though I took some specimens in a large cement-lined reservoir on Kuala Selangor Hill, far above high tide, though near the sea. Whether the Malayan *ludlowi* is identical with the form described by Ludlow from fresh water in the Philippines,



only a study of considerable numbers of the two types will show.

Of the types of *rossi* included in the table, *A. rossi* var. *indefinitus* and *A. rossi* type Giles refer to type *indefinitus* and type Giles compared above. "Known" means that forms were identified by examination of both larva and adult, and "probable" refers to such specimens as were identified by adult characters alone taken in connection with the character of the breeding place. The coast *indefinitus* all came from brackish water, and the character of the adult, of the breeding places, and of such larvæ as were examined makes it practically certain that they were all of the *indefinitus* type and not mixed with type Giles. This is the more likely, since practically all came from one locality where type Giles was not found. The inland "probable" is also almost surely of the *indefinitus* type, since all were collected in the small muddy pools where, as stated above, type Giles was never found. The Giles inland "probable" is less certain, but of a large series of larvæ subsequently collected from the same habitat, practically all were of type Giles on microscopical examination. The mixed group needs no comment. The less certain forms were included, since all were *rossi*.

No other species needs special comment except *hunteri*, a form recently described by Strickland<sup>3</sup> and closely allied to *A. separatus* of Leicester.

The columns in Table II under dissection of salivary glands include practically none dissected before the tenth day after feeding on the gamete carrier. The percentage with infected salivary glands is based only on specimens with infected gut. Practically every specimen with sporozoites in the salivary glands had oöcysts, empty or otherwise, in the mid-gut, and it is believed that the percentage of gut-infected specimens that showed sporozoites in the glands gives a more definite idea of the tendency of a species once infected to form sporozoites than would a percentage based on all dissections, many of them negative. From the percentage of dissections with ova well developed (the last column of the table), dissections of specimens caught in the adult stage are excluded, a large percentage of which already had ova well formed at the time of first feeding. The *rossi* type *indefinitus* and type Giles compared in the small table appended at the bottom are included in the body of the table as well.

<sup>3</sup> Indian Journ. Med. Research, 4, 2.

TABLE II.—*Anophelines infected in the laboratory. Controlled series. All dissections.*

Species of <i>Anopheles</i> .	Cages.	Mid-gut.				
		Dis- sected.	Infected.		Number with sporo- zoites.	Average zygotes per posi- tive mid- gut.
				Per cent.		
<i>A. ludlowi</i> .....	29	69	42	60.9	2	19.0
<i>A. rossi</i> var. <i>indefinitus</i> , coast .....	28	129	31	24.0	1	12.1
<i>A. rossi</i> var. <i>indefinitus</i> , inland "known" .....	6	85	8	9.4	a 1	3.3
<i>A. rossi</i> var. <i>indefinitus</i> , inland "probable" .....	3	42	2	4.8	0	2.0
<i>A. rossi</i> type Giles, inland "known" .....	7	113	37	32.7	b 4	6.6
<i>A. rossi</i> type Giles, inland "proba- ble" .....	19	159	45	28.3	16	9.6
<i>A. rossi</i> type Giles, <i>indefinitus</i> , in- land mixed .....	9	160	25	15.6	4	3.3
Total all <i>rossi</i> .....	72	668	148	21.5	26	7.9
<i>A. kochi</i> .....	11	20	9	45.0	0	c 25.2
<i>A. aconitus</i> .....	4	6	4	66.7	1	34.5
<i>A. fuliginosus</i> .....	5	9	3	33.3	1	4.0
<i>A. maculatus</i> .....	4	10	7	70.0	0	5.3
<i>A. kawari</i> .....	4	14	10	71.4	1	5.2
<i>A. umbrosus</i> .....	21	143	25	17.5	d 5	3.6
<i>A. hunteri</i> .....	4	8	1	12.5	0	1.0
<i>A. barbirostris</i> .....	10	107	3	2.8	1	7.0
<i>A. sinensis</i> .....	6	29	1	3.4	0	3.0
Total .....	e 108	1,103	253	22.9	37	10.6

COMPARATIVE SERIES. INCLUDED IN THE ABOVE.

<i>A. rossi</i> type <i>indefinitus</i> .....	5	73	8	11.0	b 1	3.3
<i>A. rossi</i> type Giles .....	5	94	35	37.2	3	6.9
Total .....		167	43	25.6		6.5

<sup>a</sup> Sporozoites apparently abnormal.<sup>b</sup> One "very many" counted as 50.<sup>c</sup> Two "very many" counted as 50 each.<sup>d</sup> In three cases possibly infected before exposure to gamete carrier.<sup>e</sup> Subtracting those counted twice.



TABLE II.—*Anopheles* infected in the laboratory. Controlled series. All dissections—Continued.

Species of <i>Anopheles</i> .	Salivary glands, dissections 10 days after feeding.				Dissections with ova well developed. <sup>a</sup>
	Dissections.	With sporozoites.	Dissections with mid-gut positive.		
			Number.	Infected salivary glands.	
<i>A. ludlowi</i> .....	23	3	13	23.1	48.4
<i>A. rossi</i> var. <i>indefinitus</i> , coast .....	37	0	12	0.0	7.8
<i>A. rossi</i> var. <i>indefinitus</i> , inland "known" .....	16	0	1	0.0	0.0
<i>A. rossi</i> var. <i>indefinitus</i> , inland "probable" .....	0	0	0	0.0	4.8
<i>A. rossi</i> type Giles, inland "known" .....	19	2	11	18.2	30.1
<i>A. rossi</i> type Giles, inland "probable" .....	125	14	42	33.3	32.4
<i>A. rossi</i> type Giles, <i>indefinitus</i> , inland mixed .....	24	0	18	0.0	11.6
Total all <i>rossi</i> .....	221	16	84	19.0	.....
<i>A. kochi</i> .....	0	0	0	0.0	0.0
<i>A. aconitus</i> .....	1	1	1	100.0	83.3
<i>A. fuliginosus</i> .....	2	0	1	0.0	0.0
<i>A. maculatus</i> .....	0	0	0	0.0	0.0
<i>A. kawari</i> .....	6	2	4	50.0	0.0
<i>A. umbrosus</i> .....	26	2	6	33.3	81.4
<i>A. hunteri</i> .....	0	0	0	0.0	87.5
<i>A. barbirostris</i> .....	8	1	0	0.0	39.3
<i>A. sinensis</i> .....	6	0	0	0.0	82.8
Total .....	293	25	109	22.9	30.8

COMPARATIVE SERIES. INCLUDED IN THE ABOVE.					
<i>A. rossi</i> type <i>indefinitus</i> .....	4	0	1	0.0	0.0
<i>A. rossi</i> type Giles .....	18	2	10	20.0	39.5
Total .....	22	2	11	18.2	.....

<sup>a</sup> Exclusive of those caught in adult stages.<sup>b</sup> In both cases possibly infected before exposure to gamete carrier.

Most of the data found in Table II require little comment and will be summarized in connection with the species summary at the end of this paper. It may be noted, however, that of species that include a fair number of dissections *ludlowi*, *maculatus*, and *kawari* have relatively high percentages infected. *Anopheles aconitus* is also high, but the numbers are few. The brackish-water *indefinitus* is well above the fresh water, but both are below *rossi* type Giles. *Anopheles barbirostris* and *sinensis* rank low in percentage infected. *Anopheles ludlowi*, *rossi* type Giles, *aconitus*, and *kawari* show the more-marked tendency to form sporozoites in the salivary glands. *Anopheles umbrosus* may be included under those readily forming sporozoites, although the specimens with sporozoites in the salivary glands had been caught in the imago stage and may have been infected before feeding, since other specimens taken at the same time and from the same locality showed sporozoites, although not exposed to a gamete carrier. *Anopheles indefinitus* showed no sporozoites in the salivary glands, although fair numbers of the brackish-water type were dissected. *Anopheles maculatus* was largely used as a control, and none were dissected till ten days after feeding on the gamete carrier. The number of specimens of different species showing sporozoites in the mid-gut is included in the table, since the proportion of these gives us some indication of the probability that a species may under some conditions form sporozoites in the salivary glands.

Many cages, including many dissections, were done that are not included in Table II, since in these there was no control to show that the gametes were viable at the time of feeding. The relative numbers of controlled and noncontrolled cages and dissections are given in Table III.

TABLE III.—Comparison of controlled and noncontrolled series.

	Controlled.		Noncontrolled.	
		Per cent.		Per cent.
Cages.....	108	49.3	111	50.7
Dissections.....	1,103	53.2	969	46.8

In a large proportion of the experiments the blood of the gamete carrier was examined on the day of feeding and usually at the time of feeding. In a small proportion of cases the carrier was examined on the day preceding or the day following feeding. No carrier was used, in crescent carriers at least, who did not have a sufficient number of gametes to infect, or 1



per hundred leucocytes at least. Nearly all of the carriers, including those who failed to infect mosquitoes, had a much higher percentage of gametes than 1 per cent. In many cases, no doubt, it was more or less a matter of chance that no infections occurred, but that there is a difference in carriers aside from the number of gametes harbored in the blood, and that the same carrier may vary at different times, will be shown in connection with later tables, especially Tables X and XI. In a considerable percentage of the noncontrolled cages the carrier at some previous feeding had infected some of the mosquitoes exposed.

In Table IV are given the percentages of infection of the mid-gut in both controlled and noncontrolled cages. These percentages are of little value in comparison of species where numbers are small, since chance plays a large part in the result, but where numbers are large, the table may be of use in indicating in a general way the probability that mosquitoes of a given species may become infected through biting one or more of a miscellaneous lot of carriers such as might be found in a malarious community.

TABLE IV.—*Controlled and noncontrolled cages by species.*

Species of <i>Anopheles</i> .	Dis- sected.	Positive.
		<i>Per cent.</i>
<i>A. ludlowi</i> .....	96	43.4
Var. <i>indefinitus</i> , brackish water.....	344	9.0
Var. <i>indefinitus</i> , "known".....	165	4.8
Var. <i>indefinitus</i> , "probable".....	78	2.7
Type Giles, "known".....	175	21.1
Type Giles, "probable".....	306	14.7
Type Giles, <i>indefinitus</i> mixed.....	245	10.2
<i>A. kochi</i> .....	31	29.0
<i>A. aconitus</i> .....	22	18.2
<i>A. fuliginosus</i> .....	33	9.1
<i>A. maculatus</i> .....	40	17.5
<i>A. kawari</i> .....	31	32.3
<i>A. umbrosus</i> .....	240	10.4
<i>A. hunteri</i> .....	10	10.0
<i>A. barbirostris</i> .....	197	1.5
<i>A. sinensis</i> .....	64	1.6
<i>A. aitkeni</i> .....	1	0.0
Total.....	2,072	12.2

In Table V dissections are classified according to the type of gamete harbored by the carrier. Controlled series alone are included.

TABLE V.—Controlled series. Dissections according to gamete carriers.

Species of <i>Anopheles</i> .	Crescents only.		Benign tertian only.		Benign tertian plus crescents.		Benign tertian plus quartan plus crescents.	
	Dis- sected.	Pos- itive.	Dis- sected.	Pos- itive.	Dis- sected.	Pos- itive.	Dis- sected.	Pos- itive.
		<i>P. cent.</i>		<i>P. cent.</i>		<i>P. cent.</i>		<i>P. cent.</i>
<i>A. ludlowi</i> .....	61	65.6	8	25.0				
<i>A. indefinitus</i> , brackish water..	101	29.7	28	3.6				
<i>A. indefinitus</i> , "known".....	73	11.0			12	0.0		
<i>A. indefinitus</i> , "probable".....	42	4.8						
Type Giles, "known".....	113	32.7						
Type Giles, "probable".....	159	28.3						
Type Giles and <i>indefinitus</i> , mixed.....	160	15.6						
<i>A. umbrosus</i> .....	117	16.2	8	0.0	18	33.3		
<i>A. barbirostris</i> .....	82	0.0			10	20.0	15	6.7
<i>A. sinensis</i> .....	21	0.0					8	12.5
<i>A. hunteri</i> .....	5	20.0			8	0.0		
<i>A. kochi</i> .....	18	50.0	2	0.0				
<i>A. aconitus</i> .....	4	75.0			2	50.0		
<i>A. maculatus</i> .....	5	100.0			5	40.0		
<i>A. kawari</i> .....	12	83.3			2	0.0		
<i>A. fuliginosus</i> .....	3	33.3			5	40.0	1	0.0
Total.....	976	24.1	46	6.5	57	22.8	24	8.3

It will be noted in Table V that the majority of the carriers harbored crescents. Where two sorts of gametes occurred in one or more carriers in the course of a feeding experiment, there was in most cases doubt as to which kind of parasite infected the mosquitoes. However, in the case of *barbirostris* exposed to three carriers having different kinds of parasites, it seems clear that the mosquito found infected was infected with benign tertian. Well-formed sporozoites were present in the oöcysts in the gut. Only six days intervened between exposure to the quartan carrier and dissection, and only four days intervened in the case of the crescent carrier, periods of time too short for the formation of sporozoites. So it is at least highly probable that the effective gamete was from the benign-tertian carrier in which the interval between feeding and dissection was eight days. In the case of the single positive dissection in *sinensis* the evidence is less clear, but it is highly probable that the single positive mosquito found was infected with sub-tertian. Only two small oöcysts were found in the gut, these apparently not over 6 days old (measurement 22.1 and 30.6 microns in diameter, respectively), and ten days had elapsed since the last exposure to a benign-tertian or to a quartan



carrier. The arrangement of the pigment in the oöcyst resembled that of subtertian, although I have observed much variability in this character in undoubted subtertian oöcysts. The *sinensis* had been exposed to a relatively "potent" carrier, No. 1997, to be described later. It will be observed in this table that infections were obtained with both benign tertian and crescents in *ludlowi* and in var. *indefinitus* from brackish water.

In Table VI all gamete carriers are classified according to their number and the character of gamete harbored. Carriers are arranged under three columns: Those under A were known to harbor viable gametes at one feeding, at least; those under B appeared in connection with positive feedings, but were never the sole carriers under such circumstances—they must be classified as doubtful; those under C were always negative, never appearing in connection with a positive feeding.

TABLE VI.—*Number and character of gamete carriers.*

Type of parasite.	A, known to be positive.	B, doubtful.	C, negative.	Total.
Crescents.....	19	20	18	57
Benign tertian alone.....	2	9	7	18
Crescents plus benign tertian.....	1	0	1	2
Quartan.....	0	1	3	4
Total.....	22	30	29	81
Percentage of grand total.....	27.2	37.0	35.8	

The high percentages under B and C, of Table VI, are noteworthy, since they indicate the large proportion of gamete carriers who apparently harbor nonviable gametes.

In some cages the females were examined singly in test tubes after the first feeding, and those known to have taken blood were separated and given no further exposure to a carrier. In other cages, and possibly the larger number, insects were exposed twice or more to the same or different carriers and the "blooded" ones were not separated. In *rossi*, at least, there was some indication of a greater mortality subsequently among the "blooded" females, which were taken out immediately after feeding. Further, examination of test cages seemed to indicate clearly that few, if any, of the stronger females failed to take blood when given two or three opportunities, especially if the first feeding was done a day or more after emergence. Apparently few, if any, of the weaker ones that failed to take blood under these conditions lived long enough to be dissected. Of the dissections in the controlled series 282 known to have

taken blood and separated after the first feeding gave 27.7 per cent positives. Of those not separated, but as a rule given repeated feedings, 451 dissections gave 26.6 per cent positive, a percentage little below that of those known to have taken blood.

It may be of interest to know what relation, if any, the number of times mosquitoes exposed to gamete carriers bears to the percentage infected and to the average number of oöcysts per positive mid-gut.

Crescent series alone give the best basis for comparison, and such dissections, all from the controlled series, are summarized in Table VII.

TABLE VII.—*Relation of the percentage infected and the average number of oöcysts per mid-gut to the number of times exposed to a crescent carrier.*

Exposed to carrier.	Dis- sected.	Positive.	Average oöcysts per mid- gut.
		<i>Per cent.</i>	
Once .....	415	20.5	9.9
Twice .....	213	27.2	12.4
Three times .....	208	18.8	21.3
Four times .....	102	30.4	4.3
Five times .....	20	45.0	8.9
Six times .....	18	72.2	3.2
Total .....	976	24.1	11.3

Twelve species with greatly varying susceptibility to infection are included in this summary, and the probable error is great; however, the numbers are large enough to indicate that there is no very marked positive correlation of number of feedings to percentage infected or average number of oöcysts, except that in the aggregate those exposed four, five, and six times show a higher percentage of infections than those exposed fewer times.

There was little indication in any series of infection experiments of a new infection occurring in a once infected gut through a later feeding of the mosquito on a carrier. There was some variation in the size of oöcysts, but such variation could be found in insects exposed but once. Further we note in Table VII that the average number of oöcysts does not increase with the number of feedings. The most probable case of a superimposed infection was afforded by a lot of *umbrosus* collected in the adult stage. These showed a fair percentage of specimens infected previous to exposure to a gamete carrier. A lot of these were

exposed to a crescent carrier on two successive days and on each of the three following days to a benign-tertian carrier. Nine days after the first exposure they were dissected. The mid-gut of one specimen showed 9 oöcysts not segmenting and apparently about 9 days old. Sporozoites were present in the salivary glands. In the wall of the gut beside one of the immature oöcysts apparently mature sporozoites were found. There was some evidence here of a superimposed infection of the same insect.

Much evidence may be found in these experiments to indicate that the probability of infecting a mosquito depends on factors other than the number of gametes present in the carrier at the time of feeding. In Table VI, under A, we have 22 positive carriers. In fifty-eight feedings with these carriers the percentage of crescents averaged 14.8 per hundred leucocytes. In the always negative carriers, column C, thirty-four feedings gave an average of 23.2 per cent of crescents. However, 4 of these later feedings were done on insects apparently less robust, since reared from larvæ kept for some time in the laboratory. If we omit these four feedings we have an average of 15.1 per cent of crescents for the negative carriers, a percentage still slightly above that of the positive carriers.

This subject is approached in another way in Table VIII. Here dissections of the controlled lots are summarized with respect to the relation that the percentage infected and the average number of oöcysts per infected mid-gut bear to the number of crescents present in the carriers at the times of feeding. Crescent percentages are arranged in groups, and as many dissections as could be brought into these groups are included in the table. Some dissections had to be omitted, because the mosquitoes had been exposed to carriers of such widely varying percentages that they could not be brought into any group. *Anopheles umbrosus*, *barbirostris*, and *sinensis* had many negatives, and these were nearly all found in two crescent groups; so, for the sake of comparison, a second series of columns is given in which these species are omitted.

It will be noted in Table VIII that the percentage infected does not increase with the increase in percentage of crescents, but comparing groups in which there was a fair number of infected specimens, we note a steady rise in the average number of oöcysts as the gametes increase. The 75.1–100.0 group is scarcely comparable, since there were only two infected mosquitoes in that group.



TABLE VIII.—Relation of percentage injected and average number of oöcysts per infected mid-gut to percentage of crescents in carrier at time of feeding.

Crescents per 100 leucocytes.	All species.			<i>Anopheles umbrosus, barbirostris, and sinensis</i> deducted.		
	Dissected.	Infected.	Average oöcysts.	Dissected.	Infected.	Average oöcysts.
	Per cent.			Per cent.		
0.5- 2.0.....	22	50.2	3.0	17	61.7	3.0
2.1- 10.0.....	276	22.5	6.4	219	28.3	6.4
10.1- 25.0.....	298	18.5	15.9	207	27.0	15.9
25.1- 50.0.....	91	27.5	20.8	90	28.8	20.8
50.1- 75.0.....	0	0.0	0.0	0	0.0	0.0
75.1-100.0.....	16	12.5	6.5	16	12.5	6.5
Total.....	708	22.2	11.9	519	28.4	11.1

A certain carrier, patient 1997, had at one time the remarkably high percentage of 161.5 crescents. Some of the higher percentages of No. 1997 could not be included in Table VIII, since this carrier was at that time associated with carriers of much lower percentages on the same lots of mosquitoes. Some features of this carrier are of especial interest, and the data are given somewhat in detail. He was a Chinese, male, aged 24, and formerly a coolie on a rubber estate. He had been in the Federated Malay States about two years. He had a history of fever without rigor about one and one-half years previously, but had never before been in the hospital. When he came under our observation he had entered the hospital for chancre and did not know that he had malaria. Certain data on this case are included in Table IX.

This case is remarkable for the high percentage of crescents and for the lack of symptoms of malaria in the presence of many parasites, rings as well as crescents. The fever on July 1 is apparently the only symptom observed that can be ascribed to malaria. This carrier was used for many mosquitoes and was very "potent" in infecting them. Data cannot be given for all cages, since this carrier was generally used in connection with other carriers on the same cages. In one cage he was the sole carrier. This cage gave: *Anopheles kawari*, 6 positive of 7 dissected; *maculatus*, 1 positive, 1 dissected; and *fuliginosus*, 1 dissected, negative. There were four exposures to the carrier, although some mosquitoes were introduced into the cage after the first and second exposures. Gametes ranged from 161.4 per cent to 57.7 per cent. The one successful infection of *sinensis*,

if from a crescent carrier (see under Table V), was from this carrier when gametes were 13.6 per cent. Table IX shows the gradual decrease of spleen and crescents under quinine treatment.

TABLE IX.—*Certain data on crescent carrier 1997.*

Date.	Crescents per 100 leuco- cytes.	Subtertian rings per 100 leucocytes.	Temper- ature.	Remarks.
June 24	98.7	Present.....		
June 25	117.1	do .....		
June 26	147.6			
June 29			100.8	Spleen enlarged.
June 30	161.4	About 100.....	100.4	Entered our ward.
July 1	65.8	About 200.....	103.4	Quinine 30 grains. Spleen to umbilicus.
July 2	80.2	About 85.....	99.2	Quinine 30 grains and treatment contin- ued on subsequent days.
July 3	57.7	Few if any.....	100.0	
July 4			normal	
July 5				Spleen, handsbreadth. Given thymol for hookworm.
July 7	23.2			
July 8	18.0			
July 9	13.6			
July 10	9.3			Spleen two and a half fingersbreadth.
July 11	4.8			
July 12	4.4			
July 13	4.1			
July 14	3.2			
July 15	1.1			
July 16	1.4			Spleen one fingersbreadth.
July 17	0.8			
July 18	0.9			
July 20	0.4			Spleen just palpable.
July 21	0.2			
July 22	0.0			
July 23	0.3			
July 24	0.1			Discharged.

The relation of the viability of gametes to their number in the blood of the patient is of interest on purely scientific as well as on epidemiological grounds, and the data of experiments with several crescent carriers will be given in more detail. Tables X and XI contain the data of three carriers on whom a considerable number of cages of mosquitoes were fed. In Sin Tee, Table X, mosquitoes known to have taken blood were not separated, but two or more feedings were done on the same lot. In Plantation No. 1, Table X, "blooded" females only were included in all species, and there was but one exposure to the carrier.

TABLE X.—Comparison of crescent carriers *Sin Tee* and *Plantation No. 1*.  
CARRIER SIN TEE.

Date.	Crescent.				<i>A. ludlowi</i> .		<i>A. rossi</i> var. <i>indefinitus</i> , brackish water.		<i>A. umbrosus</i> , (parasites in gut).	
	First feeding.		Second feeding.		Parasites in gut.		Average oocysts per positive gut.		Dissected.	
	Per cent.	Per cent.	Per cent.	Per cent.	Dissected.	Positive.	Dissected.	Positive.	Dissected.	Positive.
November 1, 2, 3 <sup>a</sup> .....	20.7	8.1	6.2	90.0	10	Per cent.	39.6	Per cent.		Per cent.
November 3, 4, 5.....	4.4	4.9	3.5	100.0	6	100.0	24.8	33.3	b12	0.0
November 5, 6, 7.....	3.5	2.2	1.1	83.3	6	83.3	5.8		b11	0.0
November 5, 6, 7.....	3.5	2.2	1.1	0.0	b2	0.0	0.0			
November 7, 8.....	1.1	0.8		33.3	3	33.3	1.0			

Date.	Parasites in gut.							
	<i>A. barbirostris</i> .		<i>A. kochi</i> .		<i>A. sinensis</i> .		<i>A. aconitus</i> .	
	Dissected.	Positive.	Dissected.	Positive.	Dissected.	Positive.	Dissected.	Positive.
November 1, 2, 3 <sup>a</sup> .....								
November 3, 4, 5.....			b1	c 100.0				
November 5, 6, 7.....	5	0.0	b1	0.0				
November 5, 6, 7.....								
November 7, 8.....	5	0.0						



CARRIER PLANTATION No. 1.

Date.	Crescent.			<i>A. ludlowi</i> .			<i>A. rossii</i> var. <i>indefinitus</i> , brackish water.			<i>A. umbrosus</i> , (parasites in gut).	
	First feeding.	Second feeding.	Third feeding.	Parasites in gut.		Average oöcysts per positive gut.	Parasites in gut.		Average oöcysts per positive gut.	Dissected.	Positive.
				Dissected.	Positive.		Dissected.	Positive.			
December 6	Per cent.	Per cent.	Per cent.		Per cent.			Per cent.			Per cent.
December 7	19.2			11	45.5	9.0	17	11.8	3.5		
December 8	12.3			1	100.0	40.0	7	71.4	10.0	b7	0.0
December 9	8.0			3	33.3	19.0	5	60.0	16.7	b2	0.0
December 10	6.0			3	100.0	1.3	18	5.6	3.0		
December 11	7.3			3	33.3	6.0	11	27.3	1.7		
December 12	7.8			5	0.0	0.0	16	0.0	0.0	b6	0.0
December 13	4.9						16	0.0	0.0	b4	0.0
December 14	4.1						d29	0.0	0.0	b2	0.0
December 15	5.9						11	0.0	0.0	b1	0.0
December 16	2.6						14	0.0	0.0	b12	0.0
December 16	3.5			1	0.0	0.0	33	0.0	0.0	b1	0.0

<sup>a</sup> Morning feeding—all others evening.<sup>b</sup> Imagoes caught fully developed. All others were bred from larvae or pupae in the laboratory.<sup>c</sup> One oöcyst in gut.<sup>d</sup> One out of 29 imagoes caught fully developed.



It will be noted in Table X that carrier Sin Tee was capable of infecting mosquitoes even after the percentage of crescents had fallen to 1.1, while carrier Plantation No. 1 apparently ran out of viable gametes and failed to infect even when the percentage of crescents was as high as 7.8 and a fair number of mosquitoes of susceptible species were exposed, all known to have taken blood. Plantation No. 1 was probably getting more quinine than Sin Tee, but as numerous experiments have shown, quinine apparently has little effect on gametes further than to increase the rate of their disappearance.

Table XI includes similar data of another crescent carrier, patient 537. Here only one species was exposed to the carrier, *A. rossi* type Giles "probable" (see discussion preceding Table II). In part of the cages only "blooded" females, separated after one feeding, are included. Other cages were exposed twice on succeeding days, and the females known to have taken blood were not segregated. The number of "blooded" mosquitoes surviving to be dissected appears in the last column of the table, data worthy of note but of little value for comparison, since the time intervening between feeding and dissection varied from three to thirty days.

TABLE XI.—Gamete carrier 537. All *A. rossi*.

Date of feeding.	Crescents.	Times exposed.	Known to have taken blood.	In cage after feeding.	Dissected.	Positive.	Average oöcysts per positive gut.
	<i>Per cent.</i>					<i>Per cent.</i>	
February 26.....	29.9	11	All .....	76	22	18.2	4.3
February 26 and 27.....	35.2	2	.....		26	38.5	31.5
February 27.....	35.2	1	All .....	31	4	0.0	0.0
February 27 and 28.....	27.7	2	.....		3	33.3	50.0
February 28.....	27.7	1	All .....	20	2	0.0	0.0
February 28 and 29.....	22.8	2	.....		12	50.0	10.0+
March 2.....	10.8	1	All .....	27	4	25.0	2.0
March 3.....	9.6	1	do .....	7	2	0.0	0.0
March 3 and 4.....	5.1	2	.....		1	100.0	1.0
March 4 and 5.....	1.7	2	.....		13	68.2	3.0

It will be noted in Table XI that carrier 537 also retained viable gametes until the percentage of crescents had dropped to 5.1 and, at the second feeding, to 1.7. In both Table X and Table XI it will be noted that there was a tendency to decrease the average number of oöcysts as the percentage of gametes decreased, but there was no corresponding decrease in the percentage of mosquitoes infected. It will be also noted in both tables that the percentage of positives among mosquitoes



known to have taken blood is little greater than that of those exposed two or three times without segregation of those known to have taken blood.

The explanation of the great variability in the infectivity of gamete carriers independently of the percentage of gametes in the blood is not apparent. Dr. S. T. Darling has suggested that it is due to a disparity in numbers of the sexes of gametes. It may be also conceived that in the presence of a sufficient number of both sexes there is in gametes some biological factor, not apparent morphologically, which determines their fertility. Possibly gametes originating in the same oöcysts, or it may be in the same mid-gut, are less mutually fertile than gametes from more widely differing sources. Analogies will be found in other organisms. Further data will be necessary for the explanation of this problem.

In Table X may be noted the failure of *umbrosus* collected in the imago stage to become infected. Further data on the relation of the source of mosquitoes to their susceptibility to experimental infection with malarial parasites are included in Table XII, where the percentages infected and the average number of oöcysts per infected gut are compared with respect to the source of the insects used. Only controlled series and only the four species of which material was taken in the adult stage are included in the table.

TABLE XII.—*Certain species compared as to the susceptibility of material from different sources.*

Species of <i>Anopheles</i> .	Mosquitoes collected in imago stage.			Mosquitoes collected as larvæ or pupæ.		
	Dis- sected.	Positive.	Average oöcysts.	Dis- sected.	Positive.	Average oöcysts.
		<i>Per cent.</i>			<i>Per cent.</i>	
<i>A. umbrosus</i> .....	100	6.0	4.5	43	44.2	3.8
<i>A. rossi</i> var. <i>indefinitus</i> , brackish water .....	12	8.3	8.0	117	15.6	12.3
<i>A. ludlowi</i> .....	2	0.0	0.0	67	62.7	19.0
<i>A. kochi</i> .....	15	26.7	16.5	5	100.0	32.2
Total .....	129	8.5	9.2	232	41.4	14.5

Entry recorded as "very many." Here reckoned as 50 oöcysts.

It is seen in Table XII that mosquitoes collected in the adult stage show in every species a lower percentage of infection, and in the total and every species but one a lower average number of oöcysts per infected gut than mosquitoes collected as larvæ or

pupæ and subsequently bred out in the laboratory. Mosquitoes collected as adults are apparently less avid of blood than those from the other source, but this cannot wholly explain the difference in infectivity. In some lots insects taken in the imago stage were known to have taken blood, and but a small proportion became infected. A record was kept of some series of insects caught in the imago stage with regard to the proportion taking blood at one exposure. In 5 lots of *umbrosus*, 44 out of 87 once exposed took blood; in *rossi* var. *indefinitus*, brackish water, 15 out of 20; in *kochi*, 7 out of 8. As a routine, material taken as adults was kept one or two days before exposure to a carrier, in order that they might have time to digest the blood already present in the gut. They were then, as a rule, exposed several times; it is probable that the majority of them took blood on repeated exposures. Whatever the explanation, there is evidence that such insects are less susceptible to infection, and they certainly afford less favorable material for infection experiments.

The periods of time intervening between exposure to gamete carriers and dissection are summarized in Table XIII. Only controlled series are included. Since the same cage was sometimes exposed on several succeeding days, the day groups are made large, so as to include as large a proportion as possible of the dissections. In lots exposed to carriers on a series of days one cannot, of course, determine exactly the period intervening between actual infection and dissection.

TABLE XIII.—Periods intervening between feeding and dissection.

Days.	Dis- sected.	Positive.
		<i>Per cent.</i>
Three to six .....	258	27.9
Seven to nine .....	134	23.9
Ten to fifteen .....	251	33.1
Fifteen and one-half to twenty .....	87	19.5
Twenty-one to twenty-eight and one-half .....	13	30.8
Total .....	743	29.5

Specimens were often dissected in the earlier day periods in order to determine the percentage of gut infections. Where there was evidence of infection in a cage, further dissections were often postponed to a time when sporozoites might be found. This explains the relative fewness of dissection in the 7- to 9-day group as compared with the 3- to 6- and 10- to 15-day groups.

In Table XIV dissections at later periods of 101 mosquitoes are compared with respect to different stages of development of the parasites. Only positive specimens are included, and in nearly all cases at least ten days had intervened between feeding and dissection. In cases where cages had been exposed two or more times, the time in days is given as definitely as possible. For example, the entry "11-11.5" means that the mosquitoes were fed on the carrier on the evening of one day and again on the morning of the next and that 11-11.5 days intervened between those dates and dissection. All positive cases of all species dissected ten or more days after feeding are included in this table.

In Table XIV we note that the first appearance of sporozoites in the gut was eight days after feeding. The species was *rossi*, and it had been given one feeding on a crescent carrier. The 8- to 12-day sporozoites in the gut of *ludlowi* was probably benign tertian, and the 4- to 8-day sporozoites in the gut of *barbirostris* probably benign tertian as well (see under Table V). The first appearance of sporozoites in the salivary glands, twelve to twelve and one-half days, was in a group of *rossi* infected by a crescent carrier (No. 537, Table XI). The 12- to 16-day appearance of sporozoites in *kawari* followed feeding with crescent carrier 1997 (Table IX). In the case in which sporozoites were found in the salivary glands twenty-five to twenty-five and one-half days after feeding (twenty-five days after the last feeding, crescent carrier 537), sporozoites were found in the salivary glands of another specimen of the same feeding that was dissected nine days previously. In another lot fed on the same crescent carrier eleven days intervened between the first and the last finding of sporozoites in the salivary glands. We have here some data as to the length of time sporozoites may remain in the salivary glands.

We note in this table the large proportion of dissections, many of them long after the time of infection, in which only degenerate or much retarded oöcysts were found. In practically all such cases the salivary glands were examined and were always found negative. The evidence for abnormality in these oöcysts is based not only on retardation of growth, but also on their appearance. Abnormal vacuolization and watery or coarsely granular protoplasm were sometimes seen, and in some cases the oöcyst not only remained small after a long period, but also gave the impression of an encysted body. In only one or two cases were the oöcysts themselves parasitized by "black spores" or by some similar organism.



TABLE XIV.—Later stages of development of parasites. Dissections according to days after feeding.

Species of <i>Anopheles</i> .	Interval after feeding.	Oöcysts only.			Sporozoites.		Total dissected.
		Large, apparently unsegmented.	Segmenting or in prezoö-sporangial stage.	Degenerate or much retarded. No sporozoites.	In gut only.	In salivary glands.	
	<i>Days.</i>						
	8				1		1
	10	1					1
	10-11	1		1	2		4
	11	1		1			2
	11-11.5				2		2
	12			1			1
	12-12.5			1	2	2	5
	12-13			1			1
	13			1			1
	13-13.5			2		1	3
	13.5-14			1	2		3
	14			2			2
<i>A. rossi</i> , type Giles, and <i>A. indefinitus</i> mixed; mostly type Giles.	14-14.5				1		1
	15			1			1
	15-15.5			2			2
	16				1		1
	16-16.5			2	1	3	6
	17			1			1
	17-17.5			2		1	3
	18-18.5					1	1
	19-19.5					2	2
	20			1			1
	20-20.5					1	1
	21-21.5			1			1
	22			1			1
	23-23.5			1		2	3
	25-25.5					1	1
Type Giles, "known".	28-28.5			1			1
	12-13				1		1
	13-14			1	2		3
	14-17			2		1	3
<i>A. indefinitus</i> , "known".	15-16			2		1	3
	13-14				1		1
<i>A. rossi</i> , salt water, probably <i>indefinitus</i> .	11	1		1			2
	13	2	2	2	1		7
	14			2			2
	8-12			1			1
<i>A. ludlowi</i>	11			1			1
	13	3	1			1	5
	14	1	1			2	4
	15	1					1
<i>A. walkeri</i>	4-9				2		2
	6-11		1				1
	9-12	1					1
	11-14	3					3
<i>A. kasevici</i>	9-13	2					2
	12-16					2	2
<i>A. aconitus</i>	10-13					1	1
<i>A. barbirostris</i>	4-8				1		1
<i>A. fuliginosus</i>	10-13				1		1
Total		17	5	36	21	22	101

In the varieties of *rossi*, especially, the proportion of these abnormal oöcysts is large, even in cases dissected sixteen or more days after feeding. Even if we do not regard these oöcysts as degenerate, but merely much retarded in growth, the occurrence of so large a proportion of them must affect the species as a carrier of malaria, since the delay in formation of sporozoites would materially decrease the proportion of infected mosquitoes surviving to transmit infection. In all varieties of *rossi*, 34 mosquitoes exhibiting only abnormal oöcysts were found, while cases that showed sporozoites in either gut or salivary glands totaled only 33. The temperature prevailing during these experiments was high and so nearly uniform that differences in the rate of development of malarial parasites could be hardly ascribed to variations in temperature.

#### MOSQUITOES INFECTED IN NATURE

In Table XV are summarized by species the results of dissections of mosquitoes caught in the adult stage. None of these were subsequently exposed to a carrier except in the case of a lot of 14 *umbrosus*, of which three had sporozoites in the salivary glands. These fourteen were caught in the hospital of a certain rubber estate and later exposed to a carrier. They were dissected five to eight days after feeding, and three had sporozoites in the salivary glands. Since it is highly improbable that sporozoites could be formed in the salivary glands within eight days, it seems fair to reckon these three with the naturally infected lot. Other specimens of *umbrosus* caught at the same time and place and not subsequently exposed to a carrier showed sporozoites in the salivary glands.

It has seemed worth while in the table to classify the dissections of the different species according to the locality in which the mosquitoes were taken. In all cases the mosquitoes were caught in places where gamete carriers might be expected, but the degree of probability of infection varied greatly in the different localities. The probability was the least in "houses near the coast," although two positive *umbrosus* were found there at different times (at Port Swettenham). The "plantation coolie lines" were in all cases highly infected with malaria. In "hospitals" most of the collections were made in the malaria ward of a large hospital, where many cases of malaria were admitted, or in the hospital of a highly infected plantation.

The degree of development of ova is noted in the table, since this gives some indication of the age of the insect and, consequently, of the probability of its having had time to become infected. A large proportion of the insects was found lurking in buildings in the daytime, but some specimens were taken by lamplight. The latter are indicated in the column under remarks. Such insects in this series showed little development of ova, the wings were little frayed, and the indications were that most of these had freshly emerged and were, therefore, less likely to be found infected.

It will be observed in Table XV that sporozoites in the salivary glands were found only in specimens of *A. umbrosus* and *ludlowi*. In some specimens of *A. umbrosus* the sporozoites appeared abnormally thick and short, but in other specimens they were apparently normal. In the positive specimens of *ludlowi*, sporozoites, wholly normal in appearance and staining, were found in large numbers in the salivary glands, and besides, four large pigmented oöcysts appeared in the mid-gut. The single positive specimens of *maculatus* had two pigmented oöcysts apparently about 6 days old. Of the total 667 dissections of the mid-gut, oöcysts were found in 3, or 0.4 per cent; in 508 dissections of the salivary glands 7, or 1.4 per cent, were positive for sporozoites. In 167, chiefly *A. umbrosus*, the mid-gut alone was examined. In a few cases the gut showed no trace of infection, but sporozoites were found in the salivary glands. It is evidently best to examine both gut and salivary glands if one is to ascertain the total number of positives in naturally infected mosquitoes.

It will be noted that all of the specimens of *A. kawari*, *maculatus*, and *tessellatus* and a large proportion of the specimens of *aconitus*, *sinensis*, and *fuliginosus* were taken at night. As stated in the introduction to Table XV, such material apparently includes a larger proportion of recently emerged insects than does material collected in houses by day. The one positive specimen caught by lamplight, *maculatus*, had immature oöcysts only.

In the collections in which at least one infected mosquito was found at the same time and place we have the best basis for comparison of the amount of infection in different species. In this series the numbers taken under such circumstances are too small to afford a sound comparison, but the results may be given for what they are worth.



TABLE XV.—Dissections of *Anopheles* caught. Adult stage.

Species of <i>Anopheles</i> .	Type of locality.	Dissection of—				Total gut or salivary glands.		Ova.		
		Mid-gut.		Salivary glands.						
		Dissected.	Infected.	Dissected.	Infected.	Dissected.	Infected.	Well developed.	Partly developed.	Little developed.
<i>A. ludlowi</i>	Houses near coast.....	15	0	9	0			5	0	5
	Plantation.....	3	1	3	1			3	0	0
	Total.....	18	1	12	1	18	1	8	0	5
<i>A. rossi</i> , all types	Houses near coast.....	90	0	73	0			32	20	32
	Houses inland.....	29	0	23	0			10	16	3
	Plantation coolie lines.....	16	0	16	0			12	2	2
	Hospitals.....	61	0	61	0			32	14	15
	Cow stable.....	4	0	4	0			3	0	1
	Total.....	200	0	182	0	200	0	89	52	58
<i>A. umbrosus</i>	Houses near coast.....	177	0	50	a 2			38	2	29
	Plantation coolie lines.....	29	0	29	0			10	0	19
	Hospitals.....	55	1	55	b 4			22	3	27
	Total.....	261	1	134	6	261	6	70	5	75
<i>A. acoutus</i>	Houses inland.....	2	0	2	0			1	0	1
	Plantation coolie lines.....	23	0	20	0			0	1	c 20
	Hospitals.....	33	0	39	0			15	6	17
	Cow stable.....	3	0	3	0			2	0	1
	Total.....	66	0	64	0	66	0	18	7	39
<i>A. kawari</i> .....	Plantation coolie lines.....	60	0	58	0	60	0	0	4	c 56
<i>A. maculatus</i> .....	do.....	11	1	11	0	11	1	0	0	c 11



TABLE XVI.—Positive and negative *Anopheles* in four collections.

Col- lection No.	Positive.	Negative.
1	4 <i>A. umbrosus</i> .....	46 <i>A. umbrosus</i> .
2	1 <i>A. umbrosus</i> .....	7 <i>A. umbrosus</i> , 1 <i>A. rossi</i> , 1 <i>A. ludlowi</i> .
3	1 <i>A. maculatus</i> .....	2 <i>A. barbirostris</i> , 1 <i>A. sinensis</i> , 1 <i>A. tessellatus</i> .
4	1 <i>A. ludlowi</i> .....	2 <i>A. ludlowi</i> , 2 <i>A. umbrosus</i> , 5 <i>A. rossi</i> .

Of the 8 positives of different species ova were well developed in 5. In nearly every case where a positive was obtained the average percentage of well-developed ova in both positive and negative dissections was high.

#### SUMMARY BY SPECIES

*Anopheles ludlowi*.—Much evidence has been adduced by Christophers and others that indicates that *ludlowi* is an important carrier of malaria in certain coast regions. The high percentage of infections with ready formation of sporozoites observed in the experimental series described in this paper, as well as the finding of a naturally infected specimen with sporozoites in the salivary glands, would go to confirm the evidence already obtained regarding the dangerous character of this species.

*Anopheles rossi*.—The comparatively high percentage of infections observed by me in the brackish water type of var. *indefinitus* would bring this form under suspicion, although sporozoites are apparently not readily formed. Epidemiological evidence in the coast regions of the Federated Malay States is at fault, since this type of *rossi* is there so commonly associated with *ludlowi* and *umbrosus*, both known carriers.

The var. *indefinitus* collected in fresh water shows a low degree of susceptibility to experimental infection and but little tendency to formation of sporozoites. Neither experimental nor epidemiological evidence indicates that this species is an important carrier.

*Anopheles rossi* type Giles of Malaya shows a comparatively high percentage of infections in laboratory experiments, and sporozoites are readily formed. Further, as shown in the second part of this paper, this type is capable of infecting man under experimental conditions. Epidemiological evidence from other countries, India in particular, indicates that *A. rossi* Giles is rarely, if ever, a transmitter of malaria. But, as stated in the



earlier part of this paper, there is some evidence that type Giles of Malaya may differ, biologically at least, from *A. rossi* Giles of India. Certainly the local type is easily infected experimentally, while the Indian type is reported to be rather refractory. It is difficult to get satisfactory epidemiological evidence in Malaya in regard to type Giles, since it is there commonly associated with *fuliginosus*, *aconitus*, and other potential carriers. In one or two instances I have found the larva of type Giles in the same part of a lake in which *maculatus* and *kawari* were found. The immediate vicinity of a certain extensive breeding place of type Giles near Kuala Lumpur was not particularly malarious, but the people in the vicinity, chiefly Chinese, were in the habit of protecting themselves by means of bed nets. In another group of houses half a kilometer away and situated near a breeding place of *maculatus* the people protected themselves in a similar way and were comparatively free from malaria. In both cases the population was relatively stable, and possibly the introduction of a susceptible and less well-protected group of people into either place might be followed by an outbreak of malaria. Type Giles showed a marked avidity for blood in feeding experiments, and it is known to frequent dwellings. These characteristics, taken in connection with the experimental evidence, would bring this type under suspicion.

*Anopheles umbrosus*.—The evidence obtained in these experiments, both in regard to the artificially and naturally infected insects, would confirm Watson's conclusion that *A. umbrosus* is an important carrier in Malaya. The susceptibility of this species under experimental conditions is relatively low, but it may breed in immense numbers, and evidence from laboratory experiments as well as from the condition of adults caught in nature indicates that it is a relatively long-lived species. No exact experiments were made as to its power of flight, but adults were often found in considerable numbers at some distance from breeding places, so that it is probable that *umbrosus* is a strong flier.

*Anopheles aconitus*.—Stanton and James have recorded natural and artificial infection of this species. There were but few numbers in my experimental series, but the percentage of infections was high, and sporozoites occurred in the salivary glands. This species is often found in houses and readily takes blood. It may be found at considerable distances from its breeding places, and although a small mosquito is apparently capable of long flight. The evidence goes far to incriminate this species.

*Anopheles kochi*.—No special study was made of this species, and only such specimens as happened to be collected with other species were exposed to gamete carriers. A high percentage of gut infections was obtained, but none were dissected late enough to observe any formation of sporozoites.

*Anopheles fuliginosus*.—Stanton reports both natural and experimental infection of this species in specimens collected in Malaya. The numbers in my experiments were small, but in the experimental series one third of the specimens dissected was infected. Sporozoites were found in the gut only.

*Anopheles maculatus*.—In my series this species was largely used as a control of the susceptibility of other species, and I dissected none late enough to obtain sporozoites. The one gut-infected specimen found in nature has been mentioned in connection with Table XV. However, the works of Watson, Stanton, Strickland, and others have established the fact that this species is one of the most important carriers in Malaya.

*Anopheles kawari*.—The experiments in this series indicate that this species is highly susceptible to infection under experimental conditions. The percentage of gut infections was high, and sporozoites were formed in the salivary glands. None were found infected in nature, but nearly all of the specimens dissected had probably recently emerged. It is difficult to get satisfactory epidemiological evidence, since this species is so commonly associated with *maculatus*.

*Anopheles barbirostris* and *sinensis*.—Both are certainly little susceptible to infection experimentally. Only three infected insects were obtained in a large series of *barbirostris*, and only one was obtained in *sinensis*. Stanton has found zygotes in *sinensis* in nature. In view of the facts that these species may be infected with malaria, that they occur in large numbers, and that they readily visit houses and take blood from man, they cannot be wholly acquitted of carrying malaria, but the low percentage of infection and the epidemiological evidence indicate that neither species is an important carrier in Malaya.

*Anopheles hunteri*.—The number included in my experimental series is too small to show anything further than that this species may be infected.

In regard to the commoner jungle species of Malaya I have obtained no results on *aitheni* further than to prove that it will take blood when exposed to a carrier. Of those taking blood, the single one that lived long enough to be dissected was negative, but the larvæ had been long kept in the laboratory before they emerged, and *maculatus* controls bred under the same con-

ditions were also negative. *Anopheles tessellatus* was found abundantly on one occasion, both in jungle and in pools more or less exposed to the sun, but there was no opportunity of testing them on a carrier at that time. Of a small lot of larvæ obtained later, only two adult females were obtained, and both failed to take blood.

In summary, laboratory experiments can only prove the susceptibility of a species of mosquito to malaria under more or less artificial conditions and, in a large series, the approximate degree of susceptibility. However, judging from the agreement of laboratory experiments with other evidence in the case of known carriers, it may be concluded that a high percentage of infections experimentally with the formation of sporozoites in the salivary glands furnishes strong presumptive evidence against a given species. The evidence adduced in connection with *A. rossi* (Table XIV) makes it probable that some species of *Anopheles* may be readily infected with malaria parasites, but offer comparatively unfavorable conditions for their development. On the whole, the experiments included in this paper make it doubtful whether any common species of *Anopheles* in Malaya, with the possible exception of two or three jungle forms, is immune to infection and can be wholly acquitted of carrying malaria under certain conditions.

#### TECHNIC

No attempt is here made to describe the entire technic employed in this work, but it may be worth while to mention a few modifications of the usual technic that have proved especially serviceable.

Where many larvæ have to be examined, I have found a special kind of slide very convenient. Four small pieces of cork or thick cover glass are cemented to an ordinary slide in such a position as to support a cover glass, say 3/4-inch square, at the corners. These cork supports are made of such a height that a larva placed under the cover is held, but not crushed. As larvæ vary somewhat in size, it is well to have two or three sorts of slides at hand. These are best made in duplicate, in order that one may examine one or two larvæ on one slide, while an assistant is placing other larvæ on a second. It is convenient to have a cover glass that projects a little over the slide, so that it is easily caught in the fingers in transferring it to another slide. I have examined many hundreds of larvæ in this way, and I find that one can work very rapidly by this method and, judging from the subsequent development of the larvæ.



with no injury to the insect. The cover glass makes the use of higher powers convenient, and the details of the larvæ are more clearly seen than when examined without a cover.

In many of these experiments I have used as mosquito cages lantern chimneys as described by Darling. For the greater part, however, I have used the common wooden sieves sold for a few cents each in eastern markets. These were used at the suggestion of Dr. H. P. Hacker, and I have developed the idea and have found these sieves a most useful sort of cage. A hole about one inch in diameter is cut in the middle of the wooden side, and a piece of mosquito netting is tied over the open end of the sieve. When it is desired to introduce into the cage mosquitoes as they are bred out, larvæ or pupæ are placed in a wide-mouthed glass jar, over which a piece of mosquito netting or cloth is tied. A hole is cut in the middle of the cloth about the size of the opening in the side of the sieve. The sieve is then tied firmly over the jar in such a position that its lateral opening communicates with the opening in the cloth on top of the jar. If the jar is nearly filled with water, practically all of the mosquitoes on emerging will enter the sieve. One has then only to remove the sieve, place a flap of its netting over the lateral opening, and push the string down to hold it in place. The cage is then ready to be exposed to a gamete carrier, immediately or after the mosquitoes have been kept long enough to become hungry. Or it is easy to take out the mosquitoes by means of test tubes introduced through the lateral opening and examine them singly before placing them in cages for feeding.

In exposing such cages to a gamete carrier, the carrier lies on his back, the thighs or calves are moistened with a bit of wet cotton, and the cages are placed flat under them in close contact with the skin. A blanket is then placed over the lower part of the patient's body. Towels or other cloths may be tied around the cages so as to secure a better contact with the patient's skin. Several cages may be placed under the same carrier at the same time.

In my experiments feeding was usually done in the early forenoon or in the late afternoon. It was not found necessary to wait until nightfall. Mosquitoes usually fed well if exposed a day after emerging. *Anopheles rossi* often bit well within twelve or fifteen hours after the pupal stage. In one lot of 121 females of *A. rossi* over 85 per cent took blood within less than fifteen hours after emerging.

After feeding, the cages may be placed metal side down on

ordinary dinner plates filled with wet sand or merely partly filled with water. The wooden sides of the sieves keep moist, and further moisture can be secured by allowing a flap of the covering to dip into the water or by placing wet pieces of filter paper on the top. The cages in most of these experiments were placed in a meat safe, which was kept in the laboratory and carefully protected from ants.

No food is necessary if the mosquitoes are to be dissected within a few days. In the case of mosquitoes kept for longer periods, I have followed the method of Darling by feeding with a pinch of white sugar placed on moist filter paper in contact with the top of the cage. The proportion of mosquitoes thus fed that became infected with yeasts or bacteria was much smaller than among those fed on fruit juices of any kind.

For dissection the mosquitoes were removed from the cage singly by means of test tubes. The test tubes, each containing a single mosquito, were plugged and placed in a rack on the table convenient to the microscope. Each specimen was chloroformed immediately before dissection. Where positives were found in the first ones examined, the remainder were sometimes returned to the cage and reserved for dissection after the parasites had further matured.

The chloroformed mosquito may be spilled from the test tube directly on a slide and dissected immediately or after a preliminary examination under the low power of a compound microscope. Very fine sewing needles, ground to a blade at the tip and fixed in a stick, make convenient dissecting needles when nothing else is at hand. Very shallow drops of salt solution are most convenient in dissection. In order to prevent the rounding of the drops, I have used slides carefully cleaned in the ordinary way, and I have not found it necessary to use slides prepared with bile. After the gut is drawn out, I have found it convenient to remove the excess of liquid with a bit of filter paper cleanly cut at the margins. This may be manipulated with the needles under the dissecting lens. The drop is thus rendered very shallow, and the malpighian tubes once drawn back remain in position. More liquid may be added, if necessary, after the cover glass has been adjusted. If the last segment of the abdomen is left on the gut, this will not be crushed too flat by the cover glass. If one wishes to flatten the gut further, one has only to press down the cover with a needle.

With an assistant to chloroform the mosquitoes and keep a slide ready prepared for use, one can dissect rapidly. The dissection itself and the subsequent examination of the gut salivary

glands I have done personally in every case. The gut and salivary glands were always examined with a high dry lens and often with the oil immersion as well. Sporozoites were examined fresh and subsequently stained with Giemsa or some similar stain, in order to get confirmatory evidence through the staining reaction. I have made fair preparations of the gut also with the Giemsa stain. The gut is opened and spread on the slide as one would stretch a skin on a board for drying. If the gut is spread in a minimum of fluid and just at the moment of drying, the wall of the gut may be made to lie in a single layer and keep its position on the slide. After thorough drying, one may stain as with a blood film. In a proportion of cases one may get preparations in which the cells of the gut and those of oöcysts give somewhat the Giemsa values such as are obtained in thin blood films.

The sieve cages described above are convenient in collecting adult mosquitoes. Insects caught in test tubes are introduced into the cage through the lateral opening, which is easily kept closed with a flap of the covering. Such cages, covered with a moist cloth and placed in a basket, may be carried by rail or motor car for hours with little apparent harm to the insects. The cages may be placed on plates supplied with water and kept there for a day or so until the insects have digested the blood often present in the gut at the time of collection. They are then ready for dissection.

## II. EXPERIMENTAL INFECTION OF MAN WITH MALARIA BY MEANS OF ANOPHELES ROSSI

At the time these experiments were undertaken, there were no infected *rossi* available of which the larvæ had been examined, so that we lack the crucial test as to which type of malaria *rossi* was used for infecting the experimental cases. However, the evidence points very strongly to type Giles. After feeding infected mosquitoes on the experimental cases, the whole lot exposed was always examined singly in test tubes and the ones that had taken blood were immediately dissected. By this means it was known what individual mosquitoes were infected of those that had bitten the patient. The sporozoite-infected specimens that bit these experimental cases all had the broader type of terminal black band.<sup>4</sup>

Further we have seen in part I of this paper that type Giles

<sup>4</sup> Two that bit case 727 had palp ratios of 0.3 and 0.3—, respectively. The one that bit case 408 had 0.3 (see Table I).



is readily infected experimentally with formation of sporozoites in the salivary glands, while type *indefinitus* is little susceptible to experimental infection. Again the pond where the mosquitoes that were used in these experimental cases were obtained was examined repeatedly during some six months subsequent to these experiments, and nearly 99 per cent of the hundreds of *rossi* larvæ collected were of the Giles type. Such evidence, taken by itself, would not be convincing, since the anopheline fauna of a given pond often varies, especially if the water level varies greatly, but the constancy of this type in the breeding place over such a long period may have some value as confirmatory evidence.

Considering all the evidence, it is practically certain that type Giles *rossi* infected the experimental cases, but one cannot wholly exclude the possibility that one or more of the insects was of type *indefinitus*.

Both experimental cases—Chinese coolies—freely volunteered to submit to those experiments, and both had seen enough of malaria to be fully aware of the possible consequences of infection.

No. 727, male, 26 years of age, born in China, had lived in the Federated Malay States a little less than five years. About two years previous to the experiments he had had one attack of fever with rigors daily, the attack lasting about two weeks. He had no history of fever since. The spleen was normal. He had had secondary eruptions of syphilis about one year previously. As shown in Table XVII, a daily blood examination showed no parasites until the fourteenth day after exposure to infected mosquitoes.

On March 15 and 16 three attempts were made to infect No. 727, but only five mosquitoes took blood. One of these had oöcysts in the mid-gut, but none had sporozoites in gut or salivary glands. On March 17 mosquitoes that had been deprived of sugar for two days were applied and five out of eleven exposed took blood. Two of the five had sporozoites in the salivary glands. The others were negative, in both gut and salivary glands. Dissections were made immediately after feeding. These mosquitoes had been infected by crescent carrier 537 (Table XI) nineteen to nineteen and one-half days previously. Mosquitoes had been exposed twice to the crescent carrier, and on the first exposure, the evening of February 26, the percentage of crescents was 35.6.

Both of the sporozoite-bearing mosquitoes had well-developed ova, a further indication that they were of type Giles, since, in the comparative infection experiments (see discussion preceding

Table II), type Giles showed a fair percentage of cases with well-developed ova at dissection, while type *indefinitus* showed practically none, comparing insects of the same age at dissection.

Experimental case 408, male, 33 years of age and formerly a mining coolie, was born in China and had been in the Federated Malay States four years. He had no history of fever. He had had beriberi three years previously and still had the beriberi gait, but was well nourished and otherwise in good physical condition. The spleen was not enlarged. No parasites were found in the blood for seventeen or more successive daily examinations. On March 20 the mosquitoes remaining from the lot used in case 727 were exposed to No. 408, but none bit, although they had not been given food for five days. On the same date seven of another lot were tried, and none bit. On March 21 the four of the seven remaining alive were tried and two bit. The first bit feebly and took only a small quantity of blood. The second was observed to bite twice, at least, and took a fair amount of blood. On dissection the first showed no parasites, but the second had one empty oöcyst in the gut and sporozoites in the salivary glands. Of another lot of three tried on the same day one bit. It had oöcysts, apparently degenerate, in the mid-gut, but no sporozoites in the salivary glands. So it seems clear that the subject was bitten by only one sporozoite-infected mosquito and that this one bit at least twice. This mosquito was also infected from crescent carrier 537 (Table XI) sixteen to sixteen and one-half days previously. The lot of mosquitoes were exposed twice to this carrier on succeeding days. At the first exposure the percentage of crescents was 5.1, at the second, 1.7.

Various observations on these two experimental cases are compared in Table XVII.

No. 727 showed no rise of temperature until some eight hours after parasites were found in the blood, while no parasites were found in No. 408 until the third day after the rise of temperature. No. 727 showed marked symptoms, headache, severe vomiting, and on the third and fourth days after illness began, severe attacks lasting about twenty minutes characterized by convulsive symptoms, clutching of the fingers, pain in the throat, and rapid and shallow respiration. The spleen was not enlarged at any time in this case. No. 408 had practically no symptoms at all, except that he complained of headache on one day. There was marked enlargement of the spleen. Both cases made a rapid and complete recovery under quinine treatment. Temperature charts of both cases are given.

TABLE XVII.—Cases experimentally infected with malaria by *A. rossi*, compared by days.CASE 727.<sup>a</sup>

Days after infection.	Parasites.	Maximum temperature.	Symptoms.	Spleen.	Quinine.
		°F.			Grains.
1-13	Negative (daily examination).	Normal.	None	Normal	Nil.
14	Few at 11 a. m. <sup>b</sup>	100.8 <sup>c</sup>	Slight	do	Nil.
15	Increased	101.8	Marked	do	Nil.
16	Little change	103.6	do	Dullness increased	Nil.
17	Marked increased	103.2	Severe	Not enlarged	40
18	Only 1 found	99.2	do	Normal	45
19	1 (?)	Normal	Slight		40
20	Negative		Little or none		30
21	do				
22	do				
23					

CASE 408.<sup>d</sup>

1-13	Negative (daily examination).	Normal	None	Normal	Nil.
14	Negative	do	do	do	Nil.
15	do	do	do	do	Nil.
16	do	do	do	do	Nil.
17	do	100.2	do	Not palpable	Nil.
18	do	101.8	do	Palpable	Nil.
19	Few	101.6	do	Two and a half fingersbreadth.	Nil.
20	Increased	103.2	do	do	Nil.
21	Further increased	99	Slight	Umbilicus	80
22	Negative	Normal	Nil	do	40
23	do	do	do		30

<sup>a</sup> Case 727. Blood examined daily eighteen days after disappearance of parasites, negative. Quinine treatment was continued at least ten days.

<sup>b</sup> For numbers, see Table XVIII.

<sup>c</sup> Began to rise from 4 to 8 o'clock in the afternoon; 100.8° was the midnight temperature.

<sup>d</sup> Case 408. Blood examined daily twelve days after disappearance of parasites, negative. Spleen April 15 (=twenty-fifth day) slightly reduced; April 20, 3 fingersbreadth; April 25, 1.5 fingersbreadth; April 27, palpable below ribs; April 29, palpable, deep respiration only; May 2, not palpable. Quinine treatment was continued eighteen days.

Some details as to the number of parasites found on different days are given in Tables XVIII and XIX. In case 727 an attempt was made to estimate roughly the number of parasites appearing in the peripheral blood on different days. A large number of erythrocytes were counted, or rather their number estimated, in a thin preparation stained by Hasting's stain. The estimate was made by means of a disk inserted into the eyepiece



and so ruled as to divide the field into quarters and eighths. By counting fractional parts of the erythrocytes found in a field and comparing similar fields, an estimate, more or less precise,

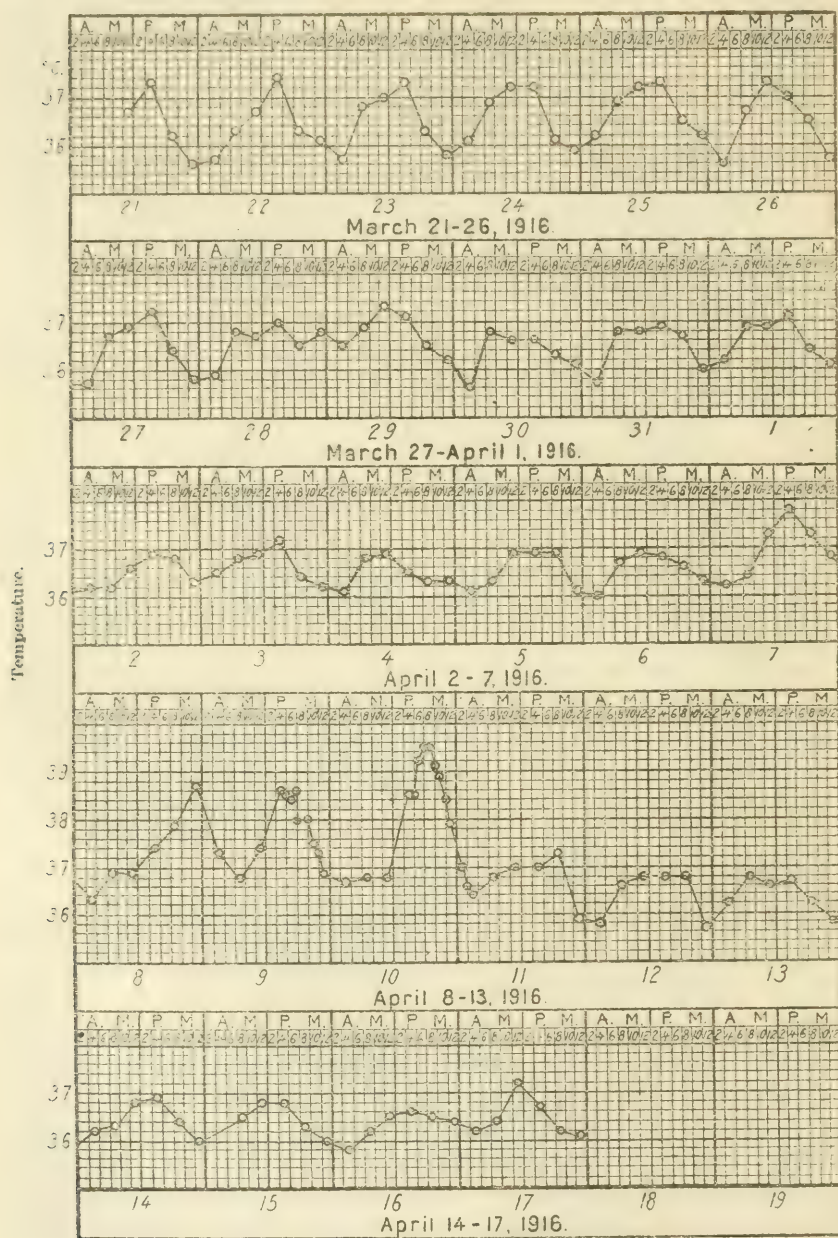


FIG. 1. Temperature chart of Thi Mue.



TABLE XVIII.—Case 727. *Parasites in peripheral blood.*

Date.	Thin film.				Thick film.				Temperature at time of taking blood samples.
	Erythrocytes estimated.	Parasites found.	Parasites per 10,000 erythrocytes.	Ratio of increase or decrease.	Leucocytes counted.	Parasites found.	Parasites per 100 leucocytes.	Ratio of increase or decrease.	
March 30.....		0				0			°F.
March 31, 11 a. m. (14th day).....	64,300	3	0.5	1.0	1,000	39	3.9	1.0	98.2
April 1, 10.45 a. m.....	47,900	37	7.7	15.4	600	237	39.5	10.1	100.2
April 2, 11.30 a. m.....	57,100	34	5.9	11.8	600	337	56.2	14.4	101.4
April 3, noon.....	18,200	39	21.4	42.8	400	756	189.0	48.9	103.2
April 4.....	+				500	136	27.2	7.0	Normal
April 5, 11.30 a. m.....		0							

<sup>a</sup> Quinine was administered at 4 o'clock in the afternoon.TABLE XIX.—Case 408. *Parasites in peripheral blood.*

Date.	Thick film.				Temperature at time of taking blood samples.
	Leucocytes counted.	Parasites found.	Parasites per 100 leucocytes.	Ratio of increase or decrease.	
April 9, 7.30 a. m. (19th day).....	( <sup>a</sup> )	5			°F. 98.6
April 10, 7.30 a. m.....	3,000	5	0.17	1	98.6
April 10, 7.15 a. m.....	1,000	5	0.50	3	<sup>b</sup> 98.2
April 12.....		0			98.0

<sup>a</sup> Long search.<sup>b</sup> Quinine was administered at 4 o'clock in the afternoon.

It will be observed in Table XVIII that, while there is some correspondence between the ratios of increase and decrease observed in No. 727 by the different methods, the results are at variance, especially on the third day. It seems probable that there was, at least, a diminution in the rate of increase of the parasites on that day. Probably the results given by the thick films are more reliable. The probable error is great in basing results on these small samples, whatever the method used. The results may give us some notion of the rate of increase or decrease of parasites, but only an approximation as to their absolute numbers in the peripheral blood.

In both cases the parasites examined carefully in thin films were evidently subtertian. No crescents were observed in either experimental case.



In summary, the evidence seems clear that these experimental cases were infected with malaria as the result of exposure to *A. rossi* infected in the laboratory. The possibility of a relapse from a former infection must be always taken into account in such experiments when performed in a malarious country. But that such relapses should follow exposure to infected mosquitoes in two cases, one occurring fourteen and the other seventeen days after exposure, would be a remarkable coincidence indeed, especially in view of the fact that both patients had been known to be free from fever many days before the experiments and that both showed the same type of parasite as that that infected the mosquitoes.

It also seems clear that a single mosquito of *A. rossi* may infect at one exposure. The fact that the case that received sporozoites from two infected mosquitoes, and, presumably, the larger dose, showed an earlier appearance of parasites and the more marked symptoms may be only a coincidence, but it is worthy of note. One can do little more than guess at the number of sporozoites injected by a single mosquito, but judging from the number of sporozoites found at dissection after feeding on the experimental cases and comparing with the numbers observed in the salivary glands of many infected mosquitoes of the same species, one would say that the effective number is a matter of hundreds rather than of thousands, and more probably a matter of scores.

It is noteworthy that the case (No. 408) that had the longer incubation period (seventeen days), the fewer parasites, and the less severe symptoms had a marked enlargement of the spleen. No. 727, with the shorter incubation period (fourteen days), the greater number of parasites, and marked symptoms, showed no enlargement of the spleen. This case had a history of a previous attack, while No. 408 had none. It is interesting to compare gamete carrier No. 1997 (Table IX), who showed enormous numbers of subtertian parasites, practically no symptoms, and a marked enlargement of the spleen. He had a history of a previous attack. This small group of three cases certainly serves well to show the great variety of manifestations observable in subtertian malaria.

#### ACKNOWLEDGMENTS

In the work included under both Part I and Part II I am under great obligations to the Government of the Federated Malay States, especially in the matter of transportation and hospital

facilities. I wish also to acknowledge my obligation for much encouragement and assistance to my colleagues in the Malaya Board, Drs. S. T. Darling and H. P. Hacker. I am also under great obligations to Dr. A. T. Stanton, of the Institute for Medical Research of the Federated Malay States, and to Dr. Malcolm Watson, of Klang. In the work included under Part II Assistant Surgeon A. E. Duraisamy furnished much assistance, especially in taking the histories and recording the clinical notes of the patients. I must further acknowledge the constant and faithful services of my laboratory assistant, Mr. Daniel Rajamoney.

## ILLUSTRATIONS

### TEXT FIGURES

- FIG. 1. Temperature chart of Thi Mue.  
2. Temperature chart of Thang Hong.





## DOES THE IRRITANT ACTION OF EMETINE HYDROCHLORIDE EXTEND TO THE KIDNEY?<sup>1</sup>

By D. DE LA PAZ and R. MONTENEGRO

*(From the Department of Pharmacology, College of Medicine and Surgery,  
University of the Philippines)*

Emetine possesses a powerful local irritant action, but its irritant effect on the kidneys and other remote organs, except the gastrointestinal tract, is not definitely known. Duckworth,<sup>(2)</sup> in 1869, observed the frequent occurrence of albuminuria in animals poisoned with emetia.<sup>2</sup> Zeff<sup>(8)</sup> found small amounts of albumin in the urine of most of his patients treated with emetine for pulmonary disease. According to Foulkrod,<sup>(3)</sup> the kidney undergoes some damage, and albuminuria results in animals given emetine. Shortly after the discovery by Vedder<sup>(6)</sup> and Rogers<sup>(5)</sup> that emetine is amœbicidal, it has been extensively used in amœbic dysentery in large doses injected repeatedly for a considerable length of time. It is surprising that this method of employing the drug has not apparently given rise to renal complications. Does the irritant action of emetine extend to the kidneys when it is administered as it is usually done in amœbic dysentery? The difficulty of obtaining a satisfactory information from the patients is self-evident. We have, therefore, carried out the present experiments on animals.

Active full-grown dogs were used for the experiments. They were given soap and water baths and rubbed dry with towels, and each was placed in a clean metabolism cage. Their diet consisted of rice, meat, and fish. Food and water were given only once in twenty-four hours at about the same time each day, but the animals were allowed each time as much as they could eat and drink. The urine for twenty-four hours was collected daily in a graduated cylinder and examined for casts and albumin. We used the test for albumin, described by Glaesgen.<sup>(4)</sup> This offers two advantages over Heller's nitric acid test; it detects the presence of a smaller pathological amount of albumin and gives a sharper reaction. When at least five successive examinations showed the absence of albumin and casts

<sup>1</sup> Received for publication August 11, 1917.

<sup>2</sup> Emetia is the alkaloid of ipecacuanha, according to Duckworth. It is probably impure emetine.

in the urine, four dogs were given hypodermic injections of a 1 per cent solution of emetine hydrochloride crystals (Paul-Merch). Baermann and Heinemann<sup>(1)</sup> consider that about 4 milligrams per kilogram of body weight are the maximal intravenous dose for man. We gave our dogs 1 milligram per kilogram of body weight. This dose corresponds to 65 milligrams or 1 grain for an adult weighing 65 kilograms; it is, according to Vedder<sup>(7)</sup> and others, usually sufficient to destroy the amœbæ in the intestine of a dysenteric patient. The injection was repeated once daily until the animals died.

Emetine caused vomiting, a rise of temperature, and hemorrhagic diarrhœa. One dog survived six injections; two dogs, seven; and one dog, eleven. Post-mortem examination revealed congestion of the internal organs, skin, and subcutaneous tissue; hemorrhages in the intestine; and in two dogs, ecchymoses at the sites of injection. Table I gives the results of daily examinations of the urine of four emetinized dogs.

The examination of the urine, as shown in the table, does not give conclusive evidence that emetine has induced inflammation of the kidneys. The appearance of albumin in the urine of dog 1 on the second day of emetine injection and in the urine of dog 3 on the fifth injection cannot be due to inflammatory changes; otherwise it should have appeared on the succeeding days and its quantity should have been increased by the subsequent injections of emetine. We cannot explain the significance of the short hyaline casts that appeared in the urine of dogs 1, 2, and 3. We also observed them in the urine of three saline controls. However, the fact that their appearance was not accompanied by albumin except in one instance (that is, on the fifth injection in dog 3) diminishes their importance as a sign of inflammatory lesion of the kidneys. This is confirmed by the examination of the sections of the kidneys. When examined histologically, the kidneys of dog 1 showed very slight acute parenchymatous degeneration; the kidneys of dog 2 showed congestion and its results, hemorrhages between the layers of the capsules, and slight if any degeneration of the tubular epithelium; the kidneys of dog 3, congestion and slight degeneration of the tubular epithelium only; and the kidneys of dog 4, very little recognizable pathological change on account of extensive post-mortem alteration. The absence of inflammation that emetine readily induces in the eye, respiratory passages, alimentary canal, and subcutaneous tissue indicates that it is not eliminated by the kidneys, or if it is, that it passes through them in very high dilution.



TABLE I.—*Examination of the urines of four emetinized dogs.*

Day of injection.	Dog No.	Urine in 24 hours.	Albumin.	Casts on 1 slide.	Remarks.
		c. cm.			
First	1	200			The amount of urine is approximate. The urine was voided outside of the cage.
	2	390	Negative	None	The amount of urine is approximate. About 150 cubic centimeters were voided outside of the cage.
	3	245	do	do	
	4	250	do	do	
Second	1	200	Trace	do	The amount of urine is approximate. About 30 cubic centimeters were voided outside of the cage.
	2	175	Negative	do	
	3	325	do	do	
	4	130	do	do	
Third	1	165	do	do	
	2	480	do	do	
	3	160	do	do	
	4	65	do	do	
Fourth	1	0			
	2	372	Negative	None	
	3	100	do	4 short hyaline	
	4	80	do	None	
Fifth	1	290	Abundant	do	Albumin probably came from the bloody stools found in the cage.
	2	160	Negative	do	
	3	170	Trace	Several short hyaline	
	4	30	do	None	Albumin probably came from the bloody stools found in the cage.
Sixth	1	90	Negative	do	
	2	125	do	do	
	3	150	do	2 short hyaline	
	4	50	do	None	The last injection was given this day. The dog was found dead on the next day.
Seventh	1	65	do	1 short hyaline	Do.
	2	230	do	None	
	3	180			Urine was mixed with bloody stools. The dog was found dead.
Eighth	2	63	Negative	1 short hyaline	
Ninth	2	125	do	Several short hyaline	
Tenth	2	120	do	do	
Eleventh	2	85			Urine was mixed with bloody stools. The dog was found dead on the next day.

Six dogs were used as controls; two received hypodermic injection of 6.7 milligrams of uranium nitrate per kilogram of body weight for two days in succession, and the remaining four received daily injections of sterile saline solution. The saline controls were autopsied after they had received the same number of injections as the emetinized animals. Saline solution did not produce hemorrhages at the places of injection nor abnormal changes in the urine and the kidneys, except the appearance of the short hyaline casts that were noted in the urine of three dogs already referred to. Their appearance cannot be ascribed to the injection of saline solution, because they were observed to appear periodically in the urine of these dogs even before the injections. Uranium nitrate caused the appearance of albumin, desquamated renal cells, granular casts, and finally anuria. When autopsied seven days after the first injection of uranium nitrate, one dog showed hemorrhage at the point of injection, anasarca, and acute tubular nephritis, while the other showed acute diffuse nephritis involving especially the tubules.

#### CONCLUSIONS

Our results show that emetine hydrochloride gave rise to congestion and slight parenchymatous degeneration of the kidneys. While in one dog the drug produced hemorrhages at the sites of injection and between the layers of the renal capsule, and at the site of injection in another dog, in no case did its irritant action extend to the parenchyma of the kidneys, although we administered it in a quantity that, when injected daily, eventually caused the death of the animals.

We wish to thank Professor B. C. Crowell, of the department of pathology and bacteriology, University of the Philippines, for examining the sections of the kidneys.

#### REFERENCES

- (1) BAERMANN, G., and HEINEMANN, H. *Münch. Med. Wochenschr.* (1913), 60, 1210.
- (2) DUCKWORTH, DYCE. *Rep. St. Bartholomew's Hosp.* (1869), 5, 244.
- (3) FOULKROD, cited by PELLINI, E. J., and WALLACE, G. B. *Am. Journ. Med. Sci.* (1916), 152, 325.
- (4) GLAESGEN. *Münch. Med. Wochenschr.* (1911), 58, 1123.
- (5) ROGERS, LEONARD. *Brit. Med. Journ.* (1912), 1, 1424.
- (6) VEDDER, E. B. *Bull. Manila Med. Soc.* (1911), 3, 48.
- (7) IDEM. *Journ. Am. Med. Assoc.* (1914), 62, 501.
- (8) ZEFF, cited by PELLINI, E. J., and WALLACE, G. B., *op. cit.*

## REVIEWS

The Diagnostics | and | Treatment of Tropical | Diseases | by | E. R. Stitt, A. B., Ph. G., M. D. | [10 lines] | second edition | revised and enlarged | with 117 illustrations | Philadelphia | P. Blakiston's Son & Co. | 1012 Walnut street | Copyright, 1917. Pp. i-xiii+1-534. Cloth, \$2 net.

The second edition of Stitt's Diagnostics and Treatment of Tropical Diseases has been brought well up-to-date by the addition of chapters on typhus fever and Rocky Mountain spotted fever in Part I, of one chapter dealing with special diagnostic problems in the tropics, and of another discussing the diagnostic value of clinical manifestations of diseases in the skin and organs of special sense. The work as a whole is to be commended as a clear and concise description of diseases incident to the tropics.

J. A. J.

Diseases | of the | Stomach, Intestines, | and | Pancreas | by | Robert Coleman Kemp, M. D. | [9 lines] | third edition, revised, with 438 illustrations | Philadelphia and London | W. B. Saunders Company | 1917 | Pp. 1-1096. Cloth, \$7 net; half morocco, \$8.50 net.

From the preface to the third edition: In view of the great value of the *x*-ray as an aid to diagnosis in the gastrointestinal tract, a special section has been devoted in this new edition to the radiography of gastric ulcer, gastric cancer, duodenal ulcer and gall-bladder disease and in addition, there are a large number of radiographs of other conditions.

Many physicians have neither time nor opportunity to devote to a practical clinical course, and next in value to this for the purpose of instruction is the employment of photography to demonstrate the methods of diagnosis and treatment. Of this, advantage has been taken.

As visceral displacements have recently assumed an important position, their symptoms, diagnosis, and treatment, notably by mechanical methods, are specially described. Typhoid fever is included in this volume on account of its intestinal complications and for the purpose of differential diagnosis.

A chapter is devoted to Diverticulitis, which has become an important subject.

The endeavor has been made to indicate as clearly as possible the conditions that call for surgical procedure.



Asthma | presenting an exposition | of | the nonpassive expiration theory  
| by Orville Harry Brown, A. B., M. D., Ph. D. | formerly assistant  
professor of medicine, St. Louis University | with a foreword by |  
George Dock, Sc. D., M. D. | professor of medicine, Washington  
University Medical School, St. Louis | Thirty-six engravings | St. Louis  
| C. V. Mosby Company | 1917 | Cloth, pp. 1-330.

The author presents in this volume an extremely comprehensive study of "Asthma." His theory of the condition, the "Nonpassive Expiration Theory," is well conceived and is clearly stated and certainly should excite further study and research. The book is based on nine years' study of asthma, and the theory, for which the author claims "that preasthmatic states can be recognized thereby and successfully combated," certainly deserves careful consideration by practitioners. Not the least pleasing feature of the work is the author's style. The work is heartily commended to the student and especially to the general practitioner, who as a rule needs something of this character to rouse him.

Doctor Brown does not play up his own theory to the exclusion of others, but gives a historical view of the subject and discusses very numerous ancient and modern theories of asthma as well as the many remedies and treatments. The latter seem to be about as numerous and varied as the theories. Almost everything from electric-light baths to diphtheria antitoxin and from resection of the fifth and sixth ribs to doses of pulverized human skull in water seems to have been used in attempts to relieve asthmatics.

The bibliographies at the ends of chapters are very full. For example, there are 470 citations following the chapter on historical observations and theories, 213 following the chapter on treatment, and 70 following clinical history, physical signs, and symptoms. The general index is a useful addition. The index to authors contains 724 names and many more entries.

The book is illustrated with a variety of engravings. Some are reproduced from kymograph records and from drawings; others are half tones from Roentgenograms. The book shows care and good taste in the printing, and the text paper is without gloss.

J. A. J. and R. C. MCG.

Traumatic Surgery | by | John J. Moorhead, B. S., M. D., F. A. C. S. | [4  
lines] | with 522 original | illustrations | Philadelphia and London |  
W. B. Saunders Company | 1917 | Pp. 1-760. Cloth \$6.50 net; half  
morocco, \$8 net.

From the preface: This book is written with the main idea

of placing in one volume the information necessary to diagnose and treat all the usual and most of the unusual effects of accident and injury.

The profession at large has become reawakened to the problems of accident surgery, and, incidentally, has come into a new relationship with the injured because of the operation of compensation and allied laws; likewise, the victims of accident, and civic, judicial, legal, and other agencies are exacting from the physician a higher grade of care and placing on him an added burden of responsibility.

The text also aims to state the measures which the writer has found most practical in his own experience, and an effort has been made to unify and standardize the treatment of such common injuries as wounds, infections, burns, and the usual fractures. It will be noted that stress is placed on the routine use of but few antiseptics, the drainage of all wounds, the immediate and complete reduction of fractures, and non-reliance upon complicated splints or those that hide the part or are irremovable.

The writer believes that open air and sunshine is the best treatment for any infected wound in any location from any source, because purulent secretion is soon checked, there are no pus-soaked or wound adhering dressings (literally pus poultices), and the comfort of the patient is measurably increased and healthy granulations and minimum scarring occur promptly. For many years now this plan has been employed, and the writer is convinced that its efficacy is best proved by the statement that skin-grafting has not been necessary since this form of aërotherapy and heliotherapy has become routine in his practice.

**Handbook of Gynecology** | for Students and Practitioners | by | Henry Foster Lewis, A. B., M. D. | [5 lines] | and | Alfred de Roulet, B. Sc., M. S., M. D. | [3 lines] | With one hundred and seventy-seven | illustrations | St. Louis | C. V. Mosby Company | 1917 | Cloth, pp. 1-452.

**Experimental | Pharmacology** | by Dennis E. Jackson, | Ph. D., M. D. | associate professor of pharmacology, Washington University | Medical School, St. Louis | With three hundred ninety original illustra- | tions including twenty-four full-page | color plates | St. Louis | C. V. Mosby Company | 1917 | Cloth, pp. 1-536.

Darwin was so repulsed by the study of geology because of the poor presentation of the subject that he resolved never again to have anything to do with it. Fortunately he revised his decision. One of the reviewer's pet theories is that the first presentation of a subject is the most important for the student. If this theory is sound, the laboratory manual holds a responsi-

ble position. Doctor Jackson's *Experimental Pharmacology* should make friends among both instructors and students. It will take a load of detail from the shoulders of the busy instructor by means of the extended and careful descriptions of methods and apparatus.

A feature that is sure to appeal to the student is the very generous use of line drawings to illustrate even the simplest instruments and apparatus. For example, the tracheal cannula is illustrated by three drawings, showing small, medium, and large-sized cannulas. Such simple and well-known laboratory equipment as the beaker, the evaporating dish, the battery jar, the scalpel, and the medicine dropper are illustrated. Four figures are given to show various sizes and styles of forceps. Each illustrated set-up, either simple or elaborate, is fully supplied with legends in large clear type. Parts of the more complicated apparatus in many cases are depicted in additional drawings. Colored plates are used to show innervation and blood vessels of various animals. Numerous reproductions of kymograph tracings show the student the kind of records that it is possible to secure. The text that goes with all of these illustrations is worthy of them. In simple, straightforward language the author tells what to do, something of what is to be looked for, and by frequent questions stimulates interest in the subject. The author writes with a frank, friendly style that is certain to win the confidence of the average student. See the following from *A Note to the Student* (p. 31):

The student will often find it necessary to carry out his work with apparatus entirely different from that described in the text and often perhaps with an equipment which is exceedingly unsatisfactory. He should by no means be discouraged thereby, for much of the most valuable experimental work of all history has been performed with crude and unwieldy apparatus, and often under most discouraging circumstances. To accomplish much with little is a sure sign of ability and the medical student who approaches the subject of experimental pharmacology at the present time will find numerous opportunities to demonstrate his aptitude in this direction.

Part one of this book begins with a Preliminary Exercise in which the organization of the class into working groups and the assignment of tables and apparatus are outlined, followed by 168 experiments.

The general anesthetics, being of fundamental importance for the progress of the course, are taken up first. Following this is a group of drugs chiefly characterized by their action on the central nervous system. After these come a series of substances possessing specific actions on some one or more parts of the involuntary nervous system. These are followed by drugs which act mainly on the circulatory system, then follow the antipy-



retics, a few miscellaneous drugs, and finally a few experiments on acids, alkalies, and some of the heavy metals.

The second part of the book contains two chapters, one on shop work and one on photography. These are chiefly of interest to the instructor, and it is advised that these be read in connection with the general preparation of apparatus, equipment, etc., for the course in pharmacology. [p. 25.]

A list of dealers in apparatus, tools, supplies, equipment, etc., and an index complete the volume.

The book is printed from large, clear type, and the experiment captions are distinguished by the use of heavy-faced type. The binding is such that the book remains open at any desired page without the necessity of breaking its back or using weights.

R. C. MCG.

Commonwealth of Australia. | Quarantine Service. | Service Publication No. 3. | The History of Small-pox in Australia, 1788-1908 | Compiled from various sources by | J. H. L. Cumpston, M. D., D. P. H., Director of Quarantine for the | Commonwealth of Australia. | Issued under the authority of the | Hon. the Minister for Trade and Customs | 1914. | By authority: | Albert J. Mullett, Government Printer. Melbourne. | Paper, pp. 1-182.

Australia has indeed been fortunate in that all available data with regard to the visitations of smallpox over the entire continent and during more than a century can be presented with such a wealth of detail within the limits of one small volume and that the entire toll of life has been only a little over 500. Its good fortune is the more notable in that its relative freedom from the disease is not due to vaccination, the number of vaccinations officially recorded being only a little over 30 per cent of the births.

Vaccination acts were passed in South Australia in 1853, in Victoria and Tasmania in 1854, and in Western Australia in 1861, requiring the vaccination of infants within six or twelve months of birth. During most of the decade from 1890 to 1899 the Tasmania Act is stated to have been a dead letter, no funds having been provided for its enforcement in certain years, while in Western Australia it appears that the Act was never thoroughly enforced, and an amendment for the relief of conscientious objectors was added in 1911. South Australia had already taken similar action in 1901. It appears that compulsory vaccination has never been required in New South Wales and Queensland, though public vaccinators were appointed, and a Vaccine Institute was long maintained at Sydney. The main

source of lymph supply since 1883 has been the Vaccine Depot at Melbourne, Victoria.

The most serious single epidemic was that that occurred at Sydney from May, 1881, to February, 1882, with a record of 151 cases, 40 of which were fatal. The expenses to the state incident to this epidemic were in excess of 400,000 dollars. Thorough compulsory vaccination would have cut it short by several months, saved lives and suffering, and nine tenths of the expense.

A most interesting epidemiologic problem is presented, but not solved, in the work. Why, with probably more than half the population of Australia unprotected by vaccination, and a large proportion of the remainder only partially protected by a single vaccination in infancy, have the epidemics of smallpox been so easily controlled. Doctor Cumpston advances the hypothesis that "the controlling factor under Australian conditions has been the absence of sufficient aggregation of population to permit of the spread of the disease so rapidly as to become beyond control." Very good as far as it goes, but not very convincing when applied to populous capitals such as Melbourne and Sydney.

J. D. LONG.

## PROCEEDINGS OF THE MANILA MEDICAL SOCIETY

REGULAR MONTHLY MEETING, NOVEMBER 5, 1917

The regular monthly meeting of the Manila Medical Society was held in the College of Medicine and Surgery on the evening of November 5, 1917, at 8.30, President Ruth presiding. There were 19 members and 2 visitors present.

The minutes of the last meeting were read and approved as read.

The application for active membership of Major J. H. H. Scudder, M. C., U. S. Army, which had been favorably considered by the council, was presented to the society for ratification. The society ratified the recommendation of the council, and the secretary was instructed to notify Major Scudder of his acceptance to active membership.

H. G. MAUL,  
*Secretary-Treasurer.*

### SCIENTIFIC PROGRAM

#### ABSCESS OF THE BRAIN IN A CHILD

By DR. MARIA MENDOZA-GUAZON

The brain of a male child, age 1.5 years, with an abscess in the central part of the left occipital lobe due to a staphylococcus infection, was shown. A well-developed pyogenic membrane was present.

#### SPECIMENS OF INTESTINAL LESIONS

By DR. B. C. CROWELL

Forty-six preserved intestinal specimens illustrating the lesions of typhoid fever and of amœbic and bacillary dysenteries were presented. These were suspended from a screen with "bulldog" paper clips for exhibition. Eighteen specimens showed the types of lesions encountered in typhoid fever, over half with ulceration of the colon (eight cases to a pronounced degree); the frequent location of lesions near the ileocæcal valve and the involvement of the appendix were noted. Seventeen specimens from cases of entamœbic dysentery were exhibited; two of these had superimposed lesions of bacillary dysentery. Duodenal ulcer was observed in two cases and gastric ulcer in



two other instances in this series. Eleven specimens were shown illustrating bacillary colitis, one case among these with superimposed cholera, a second with amœbic colitis, and a third with typhoid lesions.

#### INFECTIONS WITH COCCIDIUM AND ISOSPORA IN ANIMALS IN THE PHILIPPINE ISLANDS AND THEIR POSSIBLE CLINICAL SIGNIFICANCE

By PROF. FRANK G. HAUGHWOUT

In the light of the recent discovery in Manila of several species of coccidia, the attention of the society was directed to the possibility of the infection of human beings by these parasites of the lower animals. Several instances were cited where protozoan parasites of lower animals had been found infesting man, and attention was called to recent human infections with *Coccidium* and *Isospora* in the war zone. The paper was illustrated by microscopical demonstrations showing stages in the life cycle of both *Coccidium* and *Isospora*, the pathological changes produced by them, and comparative material illustrating the ease with which eggs of helminths may be mistaken for the cysts of coccidia and vice versa.

#### PHILIPPINE CONTACT POISONS

By PROF. E. D. MERRILL

There was a general discussion of contact poisons, not only those found in the Philippines, but those occurring in other countries. The talk was illustrated by the exhibition of herbarium specimens. The matter of those plants causing injuries by purely mechanical means was briefly mentioned, spines, bristles, etc.; the general characters of the nettle type of stinging hairs was discussed, with the structure of the hairs and their irritating contents. The fact was emphasized that all poisons of the contact type that cause violent skin eruptions, comparable to the *Rhus* or poison oak poisoning, were all from representatives of a single natural family, Anacardiaceæ, or mango family; including *Rhus*, *Semecarpus* (lacquer poisoning), *Mangifera* (mango), and *Gluta* (rengas). The poisonous principle in these plants is a nonvolatile, very permanent oil, highly irritating in character. Treatment indicated is washing the infected parts with alcohol or by treatment with lead acetate, the former acting as a solvent, the latter forming an insoluble compound with the toxicodendrol; vaseline and salves should never be used, as they merely spread the irritating oil. In conclusion, the stinging crystals or raphides of oxalate of lime,

as found in representatives of the Araceæ (*gabi* family), and certain palms were discussed; these cause intense irritation when brought in contact with the mucous membranes.

UNUSUAL LOCATION OF VACCINATION TAKE  
(DEMONSTRATION)

By DR. O. SCHÖBL

An accidental vaccination on the hand resulted from a cut due to the breaking of an ampule containing vaccine, even though the wound was cleansed with 2 per cent lysol solution. Photographs taken at intervals of three days were presented to show the development and healing of the take, which was fully developed on the ninth day. A secondary take followed. The case is of particular interest because the patient had smallpox thirty-five years ago and because of repeated unsuccessful vaccinations since that time.

R. B. GIBSON,  
*Editor of the Proceedings.*





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# THE PHILIPPINE JOURNAL OF SCIENCE

## B. TROPICAL MEDICINE

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No. 2

### BONE AND JOINT LESIONS OF YAWS WITH X-RAY FINDINGS IN TWENTY CASES <sup>1</sup>

By HERMAN G. MAUL <sup>2</sup>

(*From the Laboratory and X-Ray Department, Department Hospital,  
Manila*)

SEVEN PLATES

While attending the clinical course of instruction of the graduate school of tropical medicine and public health of the College of Medicine and Surgery, University of the Philippines, during the 1916 session, my attention was directed to the study of the painful bone and joint involvements occurring in some cases of yaws. This complaint prevailed among a great number of patients that were subsequently seen in the barrios of Las Piñas and Parañaque.

Through the courtesies of Doctors Luis Guerrero, Domingo, and Argüelles arrangements were perfected by which a group of one hundred cases of yaws was collected for study.

My work was confined to the cases suffering from bone or joint lesions. Any one who has attempted such a work among Filipinos realizes that there are certain limitations in obtaining reliable information that might materially affect one's conclusions. The first month of the work was spent in gaining the confidence of the patients by frequent visits to their homes, by furnishing free medicine, and by assuring them of no inconvenience and of a cure of the disease.

The diagnoses of these cases were made by the histories, by the clinical symptoms and manifestations, and by the demonstration of *Treponema pertenue* under the dark-field microscope

<sup>1</sup> Read, by permission of the Chief Surgeon, Philippine Department, before the Manila Medical Society, Manila, and authority granted for publication in this Journal August 6, 1917.

<sup>2</sup> First lieutenant, Medical Corps, United States Army.

in the cases where an open lesion was present, and by a careful history of those without open lesions, so as to remove any doubt as to the diagnosis.

Twenty per cent of the cases of this group of patients, as they presented themselves for treatment, suffered from bone or joint lesions. These patients were persuaded to come to the Department Hospital, Manila, for X-ray pictures and treatment. A röntgenological survey of all the bones of the body was made of each case, regardless of whether or not the patient complained of pain in the part X-rayed. Subsequent X-ray pictures were made, in order to follow the progress of the lesions under treatment.

In the majority of cases the lesions show as rarefied areas, irregularly oval or elliptical in shape with the long axis parallel to that of the bone in which the lesions are located. The size varies from the smallest discernible area to one that is two or three centimeters in length. The rarefaction presents moderately well-defined borders separating it from the unaffected bone and varies in translucency from the slightest differentiation of unnatural transparency to one simulating a perforation. Most of the lesions appear to originate in the interior of the bone, while a number can be seen as small excavations on its outer surface. When the lesion is on the surface of the bone, the periosteum is usually destroyed, but occasionally the cortex shows thickening, and the periosteum is separated from the bone. In two cases of this series there is a general thinning of the cortex of the bone and a loss of the cancellous-tissue appearance. About two per cent of the cases show a nodular type of lesion, evidenced by swelling over the surface of the bone, with a localized thickening of the cortex, which sooner or later in the course of the disease shows rarefaction in its center.

In the chronic lesions marked irregularity of the bony outline is evident, and the picture characteristic of the earlier lesions is more or less lost. The bone as a whole becomes deformed, and the growth of the bone is interfered with both in length and breadth. This dwarflike picture is most frequently noticed in the cases showing the lesions in the epiphyses. Within the joints the destruction is most frequently seen on the parts of the articular surfaces most exposed to trauma, as oval or irregularly shaped excavations, making the outline of the articular surface rough and uneven. It is concluded from this series of cases that the joint pains complained of are due in most part to the presence of the lesions on the articular surfaces.

With the exception of the 2 per cent of cases showing as a swelling over the surface of the bone, the X-ray picture is different from the bone lesion of syphilis, in that: (1) The periosteal proliferation is absent, and (2) the thickening of the cortex of the bone is absent. Also, in the 2 per cent of cases where thickening of the cortex is present, the thickening remains localized, does not tend to extend along the whole length of the bone, and sooner or later shows rarefaction in the center of the lesion.

The bone lesion of yaws may simulate (1) tuberculous or septic central abscess, (2) gumma, (3) hydatid cyst, (4) benign cyst, (5) fibrous osteitis, (6) enchondroma, (7) endothelioma, (8) secondary carcinoma, (9) myeloma, and (10) sarcoma. The differential diagnosis can be made only by combining the radiographic appearances with all clinical data, including the history, physical signs, and evidence of disease or tumor in other parts of the body.

Summarizing the findings in Table I, it is seen that in 20 cases of bone lesions in yaws:

1. The shaft of the bone is the most frequent location of the lesion and shows involvement in 80 per cent of the cases.
2. The epiphyses or articular surfaces are involved in 20 per cent of the cases.
3. The tibia is the bone most frequently involved (40 per cent of this series).
4. The order of frequency of occurrence of the lesions in the other bones is as follows:
  - (a) Tarsal bones, 40 per cent (75 per cent of these lesions occur in the os calcis).
  - (b) Fibula, 35 per cent.
  - (c) Phalanges of feet and hands, each 30 per cent.
  - (d) Metatarsal bones, metacarpal bones, and radius, each 20 per cent.
  - (e) Patella and humerus, each 15 per cent.
  - (f) Femur and ulna, each 10 per cent.
  - (g) Carpal bones, ribs, sternum, and pelvic bones, each 5 per cent.
  - (h) In the bones not mentioned no lesions were found.
5. There is no constant relation of the location of the external lesion to the bone lesion.
6. The order of frequency of occurrence of the lesions in the joints is as follows: Knee, finger, ankle, and elbow.
7. The lesions are multiple in 75 per cent of the cases, the greatest number being one hundred thirteen.
8. The time between the appearance of the primary lesion and bone lesions varies from six months to nine years, with an average of two and eight-tenths years. In this series 45 per cent of the cases showed bone lesions one year; 15 per cent, two years; 5 per cent, three years; 10 per cent, four years; 5 per cent, five years; 15 per cent, six years; and 5 per cent, nine years after the appearance of the mother yaw.



TABLE I.—Site of lesion, bones involved, interval between appearance of primary and bone lesions, and ages of patients in twenty cases of *gams*.<sup>a</sup>

	Case No.																			Total.
	1	2	3	4	5	6	7	8	9	10	11	12	20	21	22	23	24	25	30	31
Phalanges of hand	1		1					1										1		6
Metacarpus	1		1			1		1							1					4
Carpus			1																	1
Ulna			1					1												2
Radius	1		1				1	1												4
Humerus			1					1										1		3
Scapula	1																			1
Sternum																1				1
Clavicle																				0
Ribs			1																	1
Vertebra																				0
Pelvic bones			1																	1
Femur			1					1												2
Tibia			1		1	1		1				1		1	1					8
Fibula	1	1						1					1	1	1			1		7
Patella	1		1					1												3
Tarsus			1												1					2
Os calcis		1			1				1				1		1		1			6
Metatarsus	1		1	1								1								4
Phalanges of feet	1		1				1	1		1										6
Shaft	1	1	1	1	1	1	1	1	1	1	1	1		1				1	1	16
Epiphyses	1		1					1							1					4
Total lesions	19	5	52	2	2	10	8	113	1	1	2	2	2	6	18	1	1	2	1	2

Appearance of primary lesion.....	1909	1915	1911	1915	1914	1915	1911	1916	1911	1916	1909	1916	1912	1912	1905	1910	1913	1910	1915
Appearance of bone lesion.....	1910	1916	1912	1916	1916	1916	1912	1916	1912	1916	1915	1916	1917	1917	1914	1916	1917	1916	1916
Years between appearance of primary and bone lesions.....	1	1	1	1	2	1	1	$\frac{1}{2}$	3	2	6	1	4	5	2	6	4	6	1
Age.....years..	7	10	15	7	23	50	28	27	16	7	36	7	15	17	18	14	6	19	8

<sup>a</sup> Each individual bone has been tabulated but once under its respective heading, whether or not the lesion appeared in the same bone on the opposite side of the body.

<sup>b</sup> Average of years between appearance of primary and bone lesions.

Fifty per cent of the cases of this series were under 15 years of age; 75 per cent, under 20 years of age; and 90 per cent, under 30 years of age.

In the observations made by McCarthy,<sup>(17)</sup> in 1906, on the prevalence of tertiary lesions in defined localities and among certain classes of people, he gives the frequency of their occurrence and their description as follows:

(1) Chronic thickening of the skin on the palmar surface of the hands and soles of the feet.—The fissures in cases extended only partially through the skin, were painless and dry, and caused no further discomfort than a feeling of uncomfortable roughness over the affected parts.

In others, the cracks extended down to the muscular layer, exuding a sero-purulent discharge and were extremely tender on pressure. The sensibility of the surface of these parts is greatly diminished.

(2) Chronic indolent ulceration of various parts of the body.—These ulcers varied in size from small ones to those of the size of a hand or larger. When not associated with periostitis, the ulcers are painless and heal slowly. When multiple, the general health is greatly impaired, anemia and emaciation set in, and chronic invalidism is caused. Ankylosis caused by cicatricial contraction of extensive ulceration on the flexor aspect of joints has been observed in several cases.

(3) Periostitis and osteitis are other sequelæ frequently seen.—The shaft of the tibia, radius, and ulna are the usual sites of these complications. A swelling resembling a syphilitic node, appears over the shaft of the bones involving all the tissues covering it. This is at first painless. As the growth enlarges, the bones become thickened and the surface of the skin is glazed and purplish, and pain on pressure is present. The skin in time breaks down and troublesome ulceration results. When joints are affected, usually the knee, finger, and elbow-joints, with this variety of the disease, ankylosis results.

Necrosis of the nasal and palate bones resembling the syphilitic affection of this nature, have been seen in several cases.

(4) Cartilaginous tumors on the elbow and knee joints have been observed in nine cases. These tumors were painless, and caused no discomfort except for their size and position. They were ascribed by the sufferers to an antecedent attack of yaws.

Rat,<sup>(29)</sup> Daniels,<sup>(13)</sup> and Boissière reported cases with destruction of the nose and palate and discussed the probability of these lesions being due to yaws. Boissière also noted tibial involvement, joint swelling, and dactylitis.

Castellani<sup>(9)</sup> cites the sequelæ of two cases as follows:

Case 1. Young Singhalese girl of about 14 years of age. No history of syphilis either congenital or contracted: five years of age suffered, together with all other members of the family, from yaws and was treated in a Government Hospital from which she was discharged cured a few months later. She remained in till four months ago when she noticed a slight indolent swelling on the right leg which increased in size and finally broke out leaving a rather large ulceration. Two months later when I examined



her, several ulcers were present in both legs, of irregular shape, thin margins, rather deep and without much secretion; the left tibia was arching forward; moreover on one of the ribs an indolent gumateous-like swelling was present. In the secretions of the ulcers no spirochaetes were found. The girl has been treated with potassium iodide and the ulcers have healed leaving large whitish irregular scars.

Case 2. Singhalese girl about 11 years of age. Sister of the previous patient. No history of syphilis; genital organs intact. Five years ago she suffered from yaws at the same time as her sister. She recovered and remained in good health until three months ago when an ulcerative process developed on the soft palate which at the time I examined her, had already destroyed the uvula. The patient presented the thickening of the metacarpal bones and phalanges which had caused a certain distortion of the right hand. The potassium iodide treatment was begun two months ago, and the patient is rapidly improving, the ulcerative process of the palate being already arrested and healed. No spirochaetes were found in the ulcer.

Ashburn and Craig<sup>(1)</sup> cite experiments produced by Neisser, Baermann, and Halberstädter<sup>(18)</sup> where three monkeys (*Macacus cynomolgus*) were inoculated subcutaneously with the bone marrow from a monkey (*Macacus cynomolgus*) infected with frambæsia, with the result that one of the three inoculated with bone marrow developed the disease after an incubation period of forty-four days.

It is very evident that the majority of bone and joint lesions of yaws is the result of a general infection. The explanation of the peculiar selective bone manifestations in some cases may be similar to that of the various manifestations of syphilis due to variations in strains.<sup>(18, 21, 22, 23, 31)</sup> The experiments attempted by me to reproduce the bone lesions in animals have been so far unsuccessful.

In the treatment of these cases the Castellani<sup>(5, 6)</sup> mixture was used according to directions, except that a small amount of glycerin was added to improve the taste and so get the patients to take the treatment consistently.

TABLE I.—Castellani's mixture in the treatment of yaws.

	Quanti- ty.
Tartar emetic .....	grains .. 1
Sodium salicylate .....	do .. 10
Potassium iodide .....	drachm .. 1
Sodium bicarbonate .....	grains .. 15
Water q. s .....	ounce .. 1

Salvarsan was used in three of the cases, two of which received 0.4 gram, while the third received 0.2 gram, given intravenously.

In the observation of these cases, over a period of five months,

the effect of the treatment on the regeneration of the bone at the sites of the bone lesions was studied by radiographs taken at monthly intervals as nearly as was practicable. In every case the clinical and subjective symptoms disappeared long before the radiographs showed the bone lesions to have disappeared.

The histories of the most important cases are as follows:

#### CASE 1

B. U., Filipino, 8 years old. The primary lesion was on the right leg, while the patient was still a nursing baby. The secondary lesions appeared soon afterward and were most manifest on the hands and about the mouth. The secondary lesions gradually disappeared without treatment, but the mother yaw remained for over a year. Five years later the proximal phalanx of the index finger of the right hand became swollen and enlarged. Soon the adjacent fingers became similarly involved, but the patient stated that the fingers were not painful. On February 10, 1917, the X-ray pictures showed a total of nineteen bone lesions including those on the articular surfaces. The Castellani treatment was given in one-half the adult dose, but the patient soon complained of gastric disturbance and headache. The amount was then reduced to one-fourth the adult dose. After five months the bone lesions showed definite improvement, and considerable regeneration of the bones had taken place.

#### CASE 2

M. S., Filipina, 10 years old. The primary lesion was on the left leg in 1915. The secondary eruption, which appeared six weeks later, gradually disappeared after the third month without treatment. In August, 1916, she complained of pain in the left leg, which condition persisted until she was seen in February, 1917. The X-ray pictures at this time showed one lesion in the lower part of the tibia and four in the os calcis. Further observation of this case was not possible.

#### CASE 3

P. G., Filipina, 15 years old. The primary lesion appeared on the left leg in October, 1911. This lesion improved without treatment, but did not completely heal. The secondary eruption appeared three weeks later and was most marked upon the feet. Other lesions were scattered about the face, anus, and vulva. After one and one-half years the eruption had disappeared except from the lower extremities. It was elicited that severe rheumatoid pains involving all the joints developed about six months after the appearance of the primary lesion and persisted

during the next four years. During this period of her illness the soft tissues of the middle finger of her left hand became contracted and the finger could not be extended, the external lesions had become large ulcers, and the bone and joints of both extremities were so painful that she suffered constantly. In 1915 she was admitted to a hospital in Manila in a helpless condition. During the two years she remained there the condition was but little relieved, and upon returning to her home she became entirely helpless from the pain she suffered. The ulcerations were deep and painful and emitted a foul odor of decomposition.

When the patient was seen in February, 1917, she weighed 22.68 kilograms (50 pounds) and was 1.1 meters (3.5 feet) in height. There were large areas of scar tissue and of ulceration involving the greater part of the lower extremities. She was badly emaciated and anæmic and cried continuously when she attempted to walk or move about. An X-ray survey of all the bones of her body was made and a total of 52 bone lesions, including those on the articular surfaces, was found. She was given the Castellani treatment in full doses three times a day, one-half hour before meals. She continued to take the treatment regularly for the next two months, but still suffered from the bone and joint pains. The X-ray pictures taken at this time showed very slight improvement of the bone and joint lesions. She was then given 0.4 gram of salvarsan intravenously. The relief of the symptoms was as marvelous as in the cases cited by Strong in his work on cutaneous yaws,<sup>(33)</sup> in 1910, and the change in the bone and joint lesions became manifest radiographically within a month's time. No more salvarsan was given, but the Castellani treatment was continued until July 1, 1917, when the X-ray pictures showed almost complete regeneration of the bone where the lesions had been. At first the lesions showed a lessened degree of translucency, then a diminution in size, and later a return of the cancellous-tissue appearance.

During the treatment she had persistent thirst, some salivation, and nasal catarrh, but no gastric disturbances.

#### CASE 4

D. S., Filipino, 7 years old. The primary lesion was on the neck, in June, 1915. One month later the mother yaw began to disappear, and a general secondary eruption followed after a short febrile period. As the secondary eruption disappeared, rheumatoid pains appeared in several of the joints.

When the case was first seen on February 10, 1917, the right



arm was flexed at the elbow and made useless by a contracture on the anterior surface of the joint. This contracture had persisted for the past year. At this time the Castellani treatment was started in one-half the adult dose, and by the end of the second week the contracture had disappeared, but the painful bone lesions persisted for some time. Röntgenograms after full five months' treatment showed a marked improvement of the lesions, but regeneration was not complete. While taking the treatment, the patient vomited on several occasions, showed marked depression on the fifth day, became salivated, and had severe catarrhal symptoms.

## CASE 5

A. G., Filipino, 26 years old. The primary lesion appeared in August, 1914. This lesion persisted about one month. One month later the secondary eruption appeared about the axilla, elbows, mouth, anus, and prepuce, and these lesions disappeared without treatment, but soon afterward contractures of the extremities and severe rheumatoid pains in the feet developed. These conditions existed for about two years, and at the time I first saw him, he was able to get around and do light work. The X-ray pictures showed bone lesions in the left os calcis, on the articular surface of the upper extremity of the left tibia, and on the phalanges of the hands. The patient was started on the Castellani treatment February 10, 1917, and by February 25 marked improvement was evident. To hasten the recovery of the case, 0.2 gram of salvarsan was given intravenously, but the case did not return subsequently and could not be followed.

## CASE 6

A. S., Filipino, 60 years old. The primary lesion appeared on the right leg on June 5, 1915. Two months later he developed the secondary eruption and a varicose condition of the veins of the middle finger of the right hand. The finger became twice its normal size and was spindle-shaped, boggy, and worm-like to the touch, but was not painful. (It is questionable if this condition had any relation to the yaws.) He complained of pain in the tibia and femur, which had existed for one year at the time he was first seen on February 10, 1917. The röntgenograms showed a total of ten lesions. Those of the phalanges of the hands showed a marked narrowing of the cortex of the bones, while the one on the upper end of the tibia was on the surface of the bone and was excavated in character. He was given the Castellani treatment, which he took regularly, and

although he stated that his pains had left him, the lesions were still evident by the X-ray after five months' treatment.

## CASE 7

C. R., Filipino, 28 years old. The primary lesion was on the right knee in September, 1911. Two months later the secondary eruption appeared, after which there were violent rheumatoid pains in the phalanges on the feet and hands. One year after the initial lesion the distal phalanges were swollen and knoblike. On February 10, 1917, the X-ray showed rarefaction of the terminal phalanges of the toes and thinning of the cortex of the phalanges of the hands. He was given the Castellani treatment, but he disliked the medicine, and the case could not be followed.

## CASE 8

F. P. (As this case is one from which the description of the bone lesion has been made, a more detailed history will be given as prepared through the kindness of Doctor Domingo, senior house physician at the Philippine General Hospital.) General data: Filipina, 27 years old. Married, housewife by occupation. Born in Parañaque, Rizal, and has lived there ever since. She came to the Philippine General Hospital during the latter part of January, 1917, complaining of pain in the bones and joints, although she also presented several sores on the face, scalp, and neck. Smokes five to seven cigarettes each day.

*Family history.*—No history of tuberculosis or syphilis. Father and mother living and well. Has seven brothers, one of whom is in the United States, while the rest are in Parañaque. All are living and well, except one, who has the same external lesions as the patient.

*Personal history.*—The patient had her first menses at the age of 13 and married when 15 years of age. She has had four children, one of whom died of *suba*,<sup>3</sup> one has *bubas*<sup>4</sup> at present, and the other two are well. Has not had any miscarriages. Her husband is living and well and has no history of venereal disease. The youngest child is still nursing the mother.

*Previous illness.*—None relevant to the present condition.

*Present complaint.*—Primary lesion started in August, 1916, as a small papule on the anterior aspect of the left ankle. It was neither painful nor itchy. It continued to grow, and about January 16, 1917, when the case was seen with Doctor Guerrero at the Philippine General Hospital, the lesion was about 2 centi-

<sup>3</sup> Infantile beriberi.

<sup>4</sup> Yaws.

meters in diameter, in the form of a large round ulcer with more or less irregular edges and elevated granulating surface, from which an abundance of serum could be expressed. Examination of this serum showed twenty or more *Treponema pertenue* to every field. One cubic centimeter of this serum was taken from this lesion for inoculation of animals. In the latter part of October, 1916, the secondary eruption appeared on the face, scalp, neck, abdomen, and vulva. When the patient was admitted to the hospital, these lesions were nearly all circular in outline, although a few of the lesions on the face were more or less elliptical. They were raised from the surface and covered by a thick, hard yellowish crust. Removal of the crust left a raw, granulating surface. Some of the lesions of the face and abdomen were flattened out and deeply pigmented at the edges.

The pains in the bones and joints started in December, 1916, and gradually became more and more intense, until she could hardly walk. The phalangeal joints of the fingers were swollen and painful. No other joints were swollen, but pressure on the bones and other joints produced intense pain. There was no fever nor headache.

Röntgenograms of her hands were at this time taken by Doctors Fernandez and Argüelles, and an abundance of lesions was present in all the bones. The Castellani treatment was given to her by Doctor Guerrero until she left the hospital. I saw the case again on February 25, 1917. The external lesions were healed, but she still complained of pains in the bones. She had continued to take the Castellani treatment rather irregularly. She was induced to take her medicine as prescribed until March 2, when it was learned that she refused to continue with the treatment. Nevertheless she readily submitted to treatment by salvarsan, and 0.4 gram was given intravenously on March 4. The X-ray pictures taken on the same day showed no changes from those taken in February at the hospital. There was a total of 113 lesions. By the end of the second week, after this injection, the patient was entirely relieved of pain. In April the X-ray picture showed a very definite regeneration of the bone at the sites of the lesions. By the early part of July only a few places could be recognized where the lesions had existed.

#### CASE 9

R. F., Filipino, 16 years old. The primary lesion occurred on the right knee in September, 1911. The secondary eruption appeared one month later and gradually disappeared without treatment. He stated that in 1914 he suffered with pains in



the joints involving the shoulders, elbows, hips, and knees and the phalangeal joints of the fingers and toes. At the time he was seen, February 10, 1917, he was entirely well, except that his right heel was painful. The X-ray pictures showed only one lesion in the os calcis. The case could not be followed.

## CASE 22

R. C., Filipino, 15 years old. The primary lesion was on the left knee in 1905. The secondary lesions appeared a few months later and persisted until 1913. No treatment was given. The secondary lesions gradually disappeared, but the mother yaw did not heal. From this time the bones and joints of the lower extremities became painful, and by August, 1916, he was unable to walk. He was in this helpless condition when I saw him on February 25, 1917. The X-ray pictures showed a total of 18 lesions, involving the os calcis, scaphoid, tibia, fibula, and the articular surfaces of the tibia and femur on the right side and the os calcis, tibia, fibula, and the articular surfaces of the bones of the knee joint on the left side. After one month's treatment by the Castellani mixture he was able to walk with considerable ease. The case failed to come for further treatment and could not be followed.

Cases 10, 11, 12, 20, 21, 23, 24, 25, 30, and 31 are of minor interest and are only referred to in Table I.

## CONCLUSIONS

1. The majority of cases of yaws with bone and joint involvement shows characteristic X-ray lesions.

2. The radiograph can be used as an additional means of differentiating yaws from syphilis, when there is involvement of the bone, and as a confirmation of the evidence that the two diseases are distinct.

3. The pains complained of in the joints are due, in most part, to the presence of the lesions on the articular surfaces.

4. Twenty per cent of patients infected with yaws develop bone or joint lesions when not treated.

5. Regeneration of the bone is complete at the site of the lesion, if the destruction has not been too great.

6. The Castellani treatment causes a gradual disappearance of the bone and joint lesions.

7. Salvarsan is a specific in these cases, and rapid regeneration of bone follows its use.

I wish to express my appreciation to Doctors Crowell, Guerrero, Fernandez, Domingo, and Argüelles for their help and

courtesies shown while doing this work and to Mr. Hallare, who acted as interpreter and furnished most of the histories of the cases.

## REFERENCES

- (1) ASHBURN and CRAIG. *Phil. Journ. Sci., Sec. B* (1907), 2, 452.
- (2) BARRET. *Path. Soc. Lond.* (1905).
- (3) BORNE. *Geneeskundeg Tijdschrift* (1906).
- (4) BOWMAN. *Phil. Journ. Sci., Sec. B* (1910), 5, 485.
- (5) CASTELLANI. *Journ. Trop. Med. & Hyg.* (1915), 18, 61.
- (6) IDEM. *Brit. Med. Journ.* (1905), 2, 1280.
- (7) IDEM. *Journ. Trop. Med.* (1906), 9, 3.
- (8) IDEM. *Journ. Ceylon Branch Brit. Med. Assoc.* (1905).
- (9) IDEM. *Arch. f. Schiffs- u. Trop.-Hyg.* (1907), 2, 19.
- (10) IDEM. *Journ. Hyg.* (1907), 7, 558.
- (11) IDEM. *Brit. Med. Journ.* (1905), 2, 1330.
- (12) IDEM. *Brit. Med. Journ.* (1905), 2, 1280.
- (13) DANIELS. *Brit. Journ. Dermat.* (1896), 8, 426.
- (14) GIMLETTE. *Journ. Trop. Med.* (1906), 9, 149.
- (15) MACLEOD. *Brit. Med. Journ.* (1902), 2, 1913.
- (16) MANSON. *Tropical Diseases*. Cassell & Company, Ltd., London, Paris, New York (1903), 324.
- (17) MCCARTHY. *Indian Med. Gaz.* (1906), 41, 53.
- (18) NEISSER, BAERMANN, and HALBERSTÄDTER. *Münch. Med. Wochenschr.* (1906), 53, 1337.
- (19) NICHOLLS. *Blue Book* (1894).
- (20) NICHOLS. *Journ. Exp. Med.* (1910), 12, 616; *Journ. Am. Med. Assoc.* (1910), 55, 216.
- (21) NICHOLS and HOUGH. *Journ. Am. Med. Assoc.* (1913), 61, 108.
- (22) NICHOLS. *South. Med. Journ.* (1912), 5, 528.
- (23) NOGUCHI. *Journ. Exp. Med.* (1912), 15, 90, 201; *Am. Med. Assoc.* (1913), 61, 85.
- (24) PERNET. *Journ. Trop. Med.* (1905), 16, 457.
- (25) POWELL. *Brit. Journ. Dermat.* (1896), 8, 457.
- (26) IDEM. *Path. Soc. Lond.* (1905).
- (27) IDEM. *Brit. Med. Journ.* (1905), 1, 715.
- (28) RAT. *Journ. Trop. Med.* (1905), 8, 252.
- (29) IDEM. *Ibid.* (1906), 9, 135.
- (30) REASONER. *Journ. Am. Med. Assoc.* (1916), 67, 1799.
- (31) RUSSEL, CRAIG, and NICHOLS. *Bull. Surg. Gen. Office* (1913), 3.
- (32) STITT. *The Diagnostics and Treatment of Tropical Diseases*. P. Blakiston's Son & Co., Philadelphia (1914).
- (33) STRONG. *Phil. Journ. Sci., Sec. B* (1910), 5, 433.
- (34) WELLMAN. *Journ. Trop. Med.* (1905), 8, 345.

# ILLUSTRATIONS

## PLATE I

- FIG. 1. *Case 3.* Multiple yaw lesions in patella and tibia. One variety of painful joints.
2. *Case 2.* Shows three lesions in the os calcis. This involvement is very frequent and makes walking difficult and painful.

## PLATE II

- FIG. 1. *Case 1.* Shows characteristic joint lesion on the articular surface of the bone involved.
2. *Case 3.* Ankylosis following chronic joint lesion of yaws.

## PLATE III

- FIG. 1. *Case 3.* Shows contracture on flexure surface of second finger of right hand. Multiple bone lesions. Large cutaneous lesion and thickening of soft tissues on the palmar surface of index finger of right hand.
2. *Case 3.* Five months later. Shows the disappearance of the contracture. A few of the bone lesions can be distinguished.

## PLATE IV

- FIG. 1. *Case 8.* Mother yaw, before treatment.
2. *Case 8.* Mother yaw, after treatment by the Castellani mixture.
3. *Case 8.* Yaws, secondary eruption, before treatment.
4. *Case 8.* Yaws, secondary eruption, after treatment by the Castellani mixture. This patient also had bone lesions that developed in less than six months after the mother yaw (see Plates V and VI).

## PLATE V

- FIG. 1. *Case 8.* Multiple lesions in the bones that showed no change after six weeks of treatment by the Castellani mixture.
2. *Case 8.* This case showed definite change within one month after the administration of 0.4 gram of salvarsan intravenously. Three months later regeneration of the bone was practically complete.

## PLATE VI

- FIG. 1. *Case 8.* Typical bone lesions of yaws.
2. *Case 8.* Regeneration of the bone at the sites of the lesions.

## PLATE VII

- FIG. 1. *Case 1.* Shows bone and joint lesions before treatment.
2. *Case 1.* Shows lesions five months after treatment by the Castellani mixture. Note lack of regeneration of bone and deformity.
3. Case to show the chronic bone lesions of yaws with deformity. Case of Drs. R. Fernandez and Argüelles.







Fig. 1. Lesions in patella and tibia. Case 3.



Fig. 2. Lesions in os calcis. Case 2.

PLATE I.





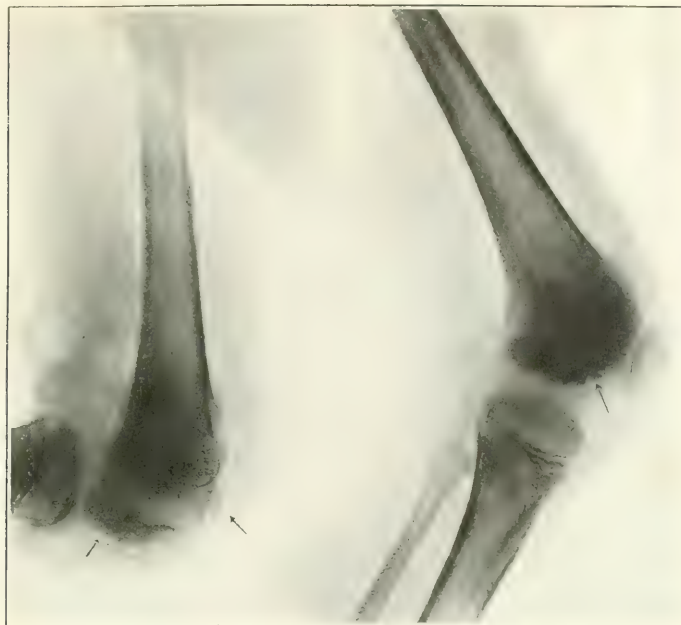


Fig. 1. Joint lesions. Case 1.

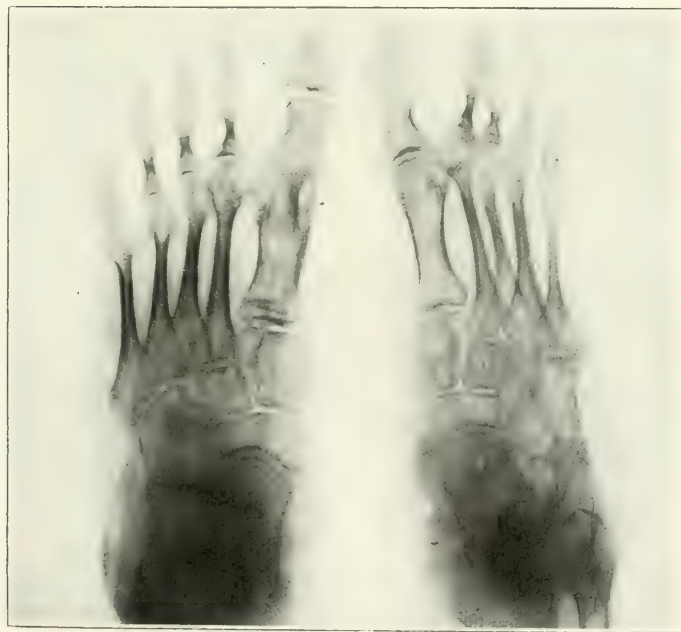


Fig. 2. Ankylosis, following joint lesion. Case 3.

PLATE II.



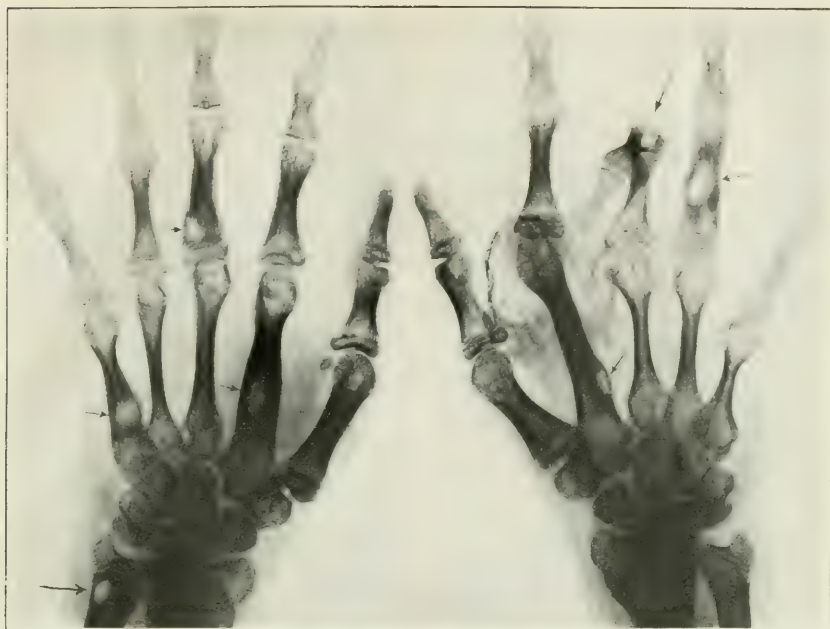


Fig. 1. Flexor contracture and multiple bone lesions. Case 3.



Fig. 2. Five months later. Case 3.

PLATE III.







Fig. 1. Mother lesion, before treatment.

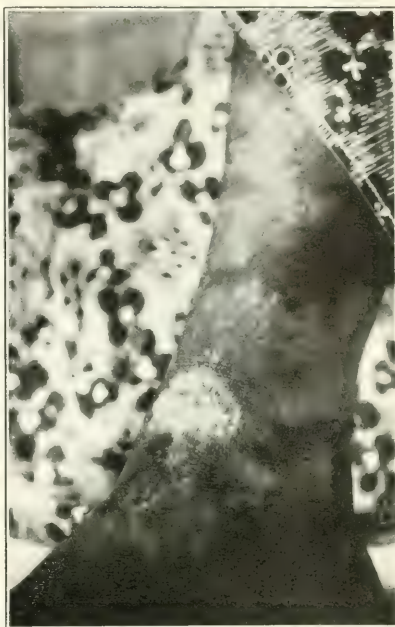


Fig. 2. Mother lesion, after treatment.



Fig. 3. Secondary eruption, before treatment.



Fig. 4. Secondary eruption, after treatment.







Fig. 1. No bone regeneration after six weeks of Castellani treatment. Case 8.



Fig. 2. Regeneration of bone of the same case after salvarsan. Case 8.

PLATE V.



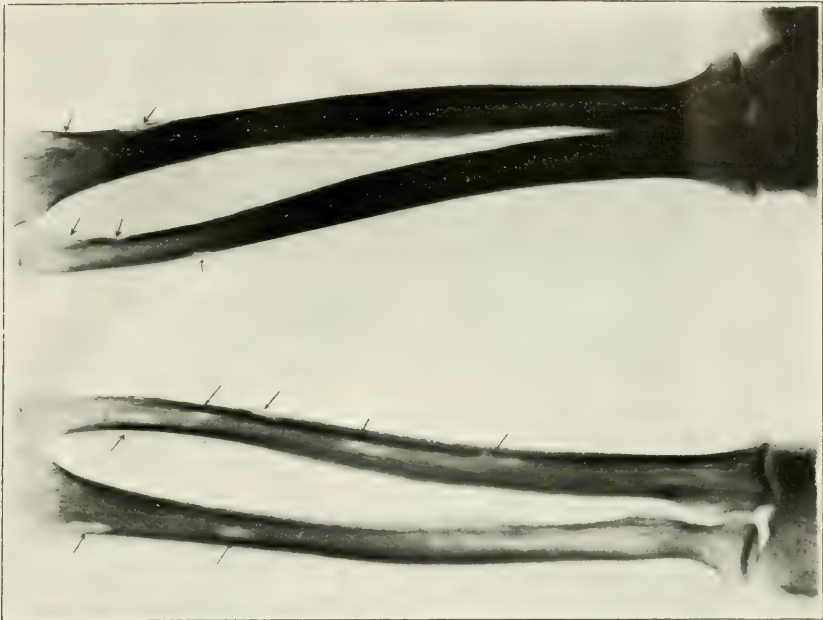


Fig. 1. Typical bone lesions of yaws. Case 8.

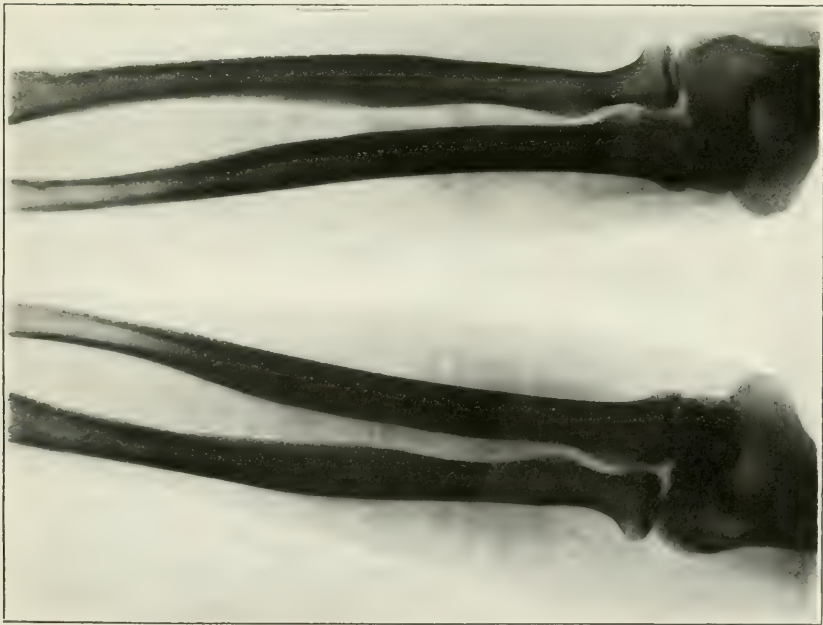


Fig. 2. Regeneration of bone at sites of lesions. Case 8. Same as above.







Fig. 1. Chronic bone and joint lesions with deformity. Case 1.



Fig. 2. Case 1 five months after treatment by the Castellani mixture.



Fig. 3. Chronic bone lesions with deformity. Case from Doctors Fernandez and Argüelles.





INFECTIONS WITH COCCIDIUM AND ISOSPORA IN ANIMALS  
IN THE PHILIPPINE ISLANDS AND THEIR POSSIBLE  
CLINICAL SIGNIFICANCE <sup>1</sup>

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University of the Philippines)

FIVE TEXT FIGURES

The recent discovery in Manila of several species of Sporozoa, presumably belonging to the order Coccidiida, which have been found infesting animals of common species, brings us face to face with the possibility of human coccidial infections, and the purpose of this paper is to present a few of the facts regarding these infections for the information of physicians who may encounter such conditions in their practice. So far as I have knowledge, no case of human coccidiosis has been reported in the Philippine Islands, but conditions supervening upon the war have led to the discovery in other parts of the world of many cases of undoubted coccidial infection, and these cases, taken in conjunction with older but less exact reports of similar infections, justify us in the belief that coccidiosis of man may in time to come be looked upon as a definite clinical entity and a condition that may crop up at almost any time or place.

In introducing the subject it seems desirable to make it clear just what is meant by coccidia. This term is rather loosely employed to describe a group of Sporozoa that are intracellular parasites, having an asexual cycle within epithelial cells where reproduction takes place by the process known as schizogony, and a succeeding sexual process involving the union of sexually differentiated gametes and spore formation within a cyst. In a general way the life cycles correspond to the classical cycle worked out by Schaudinn in the case of *Coccidium schubergi*. But it must be borne in mind that the terms "coccidium" and "coccidia" are frequently used in a rather loose and general way, and all organisms spoken of as "coccidia" are not necessarily of the genus *Coccidium*. The genus *Coccidium* is only one of a large number of genera grouped under the order Coccidiida. This order is broken up into four families

<sup>1</sup> Read before the Manila Medical Society, November 5, 1917. Received for publication October, 1917.

by Lèger, whose classification I follow: Asporocystidæ, Disporocystidæ, Tetrasporocystidæ, and Polysporocystidæ. The two genera we shall consider in this paper are the genus *Isospora* and the genus *Coccidium*, belonging, respectively, to the families Disporocystidæ, in which the oöcyst contains two sporocysts, and Tetrasporocystidæ, in which the oöcyst contains four sporocysts.

Within the past few weeks the department of medical zoölogy has received material that apparently represents six different kinds of coccidial infection. To Dr. H. Windsor Wade, of the Bureau of Science, we are indebted for calling our attention to an interesting parasite in the kidney of the guinea pig, which may prove to be identical with Seidelin's *Klossiella cobayae*, a member of the family Polysporocystidæ. Dr. Edward S. Ruth, of the department of anatomy, of the College of Medicine and Surgery, has furnished us with material showing a coccidial infection of undetermined nature in the testis of the house lizard. Dr. Elias Domingo, a graduate student in the department of medical zoölogy, has recently found a *Coccidium* and a species of *Isospora* in the intestinal tract of the house lizard. In our own department we have found *Isospora bigemina* in the intestine of kittens, while *Coccidium cuniculi* has been previously reported here in the rabbit. This latter is believed by many authorities to be identical with the parasite that has been reported in man.

This immediately raises the question as to the specificity of these parasites, and at the outset permit me to say that I consider the matter to be far from settled. To be on the safe side, however, it seems to me that we must assume that coccidial infections in animals, of a kind that is apt to be found about our houses, are a source of danger and should be so regarded until the contrary is proved. Let me cite a few examples in support of this contention.

*Trypanosoma* (*Schizotrypanum*) *cruzi*,<sup>(15)</sup> the cause of Chagas fever in South America, was first seen in its invertebrate host *Conorhinus megistus*, a blood-sucking hemipterous insect, before it was found in the blood of human beings. Lynch,<sup>(13)</sup> whose work calls for amplification, has described the rat as a host of *Entamæba histolytica*, the cause of entamæbiasis in man. Lanfranchi<sup>(11)</sup> has recently become infected with a laboratory strain of either *Trypanosoma brucei* or *Trypanosoma evansi*, both parasites of cattle. Krempf<sup>(10)</sup> has recently reported a hæmogregarine in man, while the work of Dutton and Todd,<sup>(3)</sup> Fantham,<sup>(4)</sup> Fantham and Porter,<sup>(6)</sup> and others in induced

herpetomoniasis and *Leishmania* infections is strongly suggestive of the possibility of the origin of kala-azar in man from the bites of insects harboring herpetomonads. It is finally beginning to dawn upon us, after many bitter lessons, that in the past we have paid entirely too little attention to the relations between parasites supposed to be specific to lower animals and conditions of disease occurring in man.

In dealing with the problem of coccidiosis in man, we are greatly handicapped by the vagueness and lack of information contained in reports on these infections. We have, so far as I know, no report of clinical or pathological findings in any of these cases, save the case reported by Davaine,<sup>(2)</sup> which dates back to 1858, which will lend us much help. Until recently the descriptions of the organisms found have been so incomplete as to leave us in doubt, in most cases, as to which genus was involved or, in many other cases, if, indeed, the organism was a protozoön at all. We have no literature on the intracellular phases in the cycle of the human parasite. Some of these reports are of interest to the pathologist, but they leave much to be desired from the viewpoint of the protozoölogist.

Coccidia are typically parasites of epithelial cells during their trophic phases, but Smith (17) has pointed out that they may be found between epithelial cells or even occupying subepithelial positions; however, such conditions are more or less aberrant. Infection takes place through the ingestion of matter contaminated with the spores of the parasites, and liberation of the sporozoites from the sporocysts takes place under the influence of the digestive juices of the small intestine. The sporozoites, which are minute, sickle-shaped bodies, penetrate the cell membrane of the epithelial cells and come to rest in the cytoplasm, on which they proceed to feed. As it feeds, each parasite grows, the host cell becomes enormously hypertrophied, compressing the adjacent cells, and the host-cell nucleus is crowded down to the basement membrane. Finally the trophozoite has reached the limit of size, multiple division (schizogony) takes place, the cell membrane ruptures, and a number of merozoites are set free to infect other epithelial cells. This is the so-called asexual or multiplicative phase in the life cycle, and it is repeated for a variable number of generations until conditions supervene that initiate the sexual or propagative phase, which is spoken of as sporogony.

Sporogony involves the production of sexually differentiated gametes, their union in fertilization, and the subsequent forma-



tion of cysts and spores. The significance of this process is not limited to spore formation, for with fertilization goes a complete rejuvenescence of the organism—a renewal of its vitality, which is not without its clinical significance. The busy practitioner is prone to regard this phase of the life cycle as leading up only to the infection of new hosts, and while this is an important desideratum in regard to future hosts, it yet has a very important bearing on the welfare of the original host.

The belief is very current among protozoölogists that protozoa, like other animals, are endowed with a certain potential of vitality that declines as the organism grows old. That is to say, a protozoön passes through periods that we characterize as youth, maturity, and senescence. With senescence the organism attains a degree of almost total protoplasmic stability, and unless some revitalizing agency intervenes, it dies literally of old age. In nature this rejuvenescence is brought about by the process of fertilization, which seems to be universal throughout the entire animal kingdom, and the organism issues from it endowed with a new potential of vitality with which to cope with the vicissitudes through which it must pass, which, in the case of a parasite, are many.

So it is with the coccidia. The organism passes through many asexual generations, gradually exhausting its vitality. Perhaps, also, there is the added burden of a declining food supply or other unfavorable conditions. In other words, the vitality of the parasite has become lowered, and it must have relief, else it will die.

To this condition the organism reacts by developing its propagative or sexual phase, and once this has been initiated re-infection of the original host becomes impossible except through the original channels—autoinfection ceases. Gradually the schizogonous or asexual cycle ceases, and the intracellular trophozoites become gametocytes, after which stage they are incapable of continuing the infection. The sexual phases develop, the cysts pass out with the fæces, and in time, the host is completely purged of its original infection though not necessarily immune to subsequent infections. Here the necessity for fertilization to restore the flagging vitality of the parasite has been met and has operated to bring about the self-limitation of the disease; spore formation has, in a measure, been incidental.

May this not, in part, explain the lack of information on human coccidial infections? Our information on the symptomatology of human coccidiosis is very meager. Some of the writers speak of diarrhœa, but there they stop. Chronic diarrhœa, unless

accompanied by more urgent symptoms, is frequently treated lightly by the sufferers themselves. It seems from what we know of the self-limitation of many coccidial infections in the lower animals that the same thing may occur in man and pass almost unnoticed, especially in countries where routine examinations of stools are seldom made. Furthermore coccidial cysts are frequently mistaken for the eggs of helminths and vice versa by the inexperienced microscopist.

It may be urged that the very similar life cycle of the malarial parasite and the tendency of old malarial cases to relapse apparently contradict this theory of self-limitation, but I should like to point out that in *Coccidium* and *Isospora* gametogony is completed and sporogony starts and is carried on to an advanced stage in the original host. There is encystment, and the cysts pass out and complete their development before it is possible for them to infect a new or the same host.

In *Plasmodium*, however, we have an alternation of hosts, sporogony being completed within the body of the mosquito. The sexual cycle in man is carried to the gametocyte stage only, and we have no evidence that this stage is infective to the original host. We are left here to speculate as to whether relapses of malarial fever are due to the presence of a relatively small number of trophozoites, which lie dormant in the spleen or bone marrow or which may even be free in erythrocytes in the circulation, or to some autogamous, parthenogenetic, or similar process, as Schaudinn and others have suggested.

It must not be inferred from this that all coccidial infections of this type tend toward spontaneous recovery. In many instances the infections are rapidly fatal. *Cyclospora karyolytica* gives rise to an enteritis in the ground mole, which may bring about a fatal termination in forty-eight hours, the intestinal discharges consisting almost entirely of desquamated epithelium and parasites. Young animals are prone to succumb quickly, and this should make us especially watchful in the case of children, who ordinarily come in closer contact with animals that may harbor parasites than do adults.

The most reliable information regarding coccidial infections in man comes to us from the British workers in the war zone, although Wenyon and O'Connor<sup>(23)</sup> mention human infections with *Isospora* in Egypt. Fantham<sup>(5)</sup> reports four cases of coccidial infection, "apparently *Isospora* type," found during the examination of the stools of 1,305 British soldiers in the various hospitals in the western command. These cases were all dysenterics who had become infected chiefly in Gallipoli, al-

though a few were brought in from Flanders. Woodcock (25) had previously reported similar cases that had been received from Gallipoli, which he thought were infections with *Isospora*. The cysts he saw contained one and sometimes two masses of protoplasm. Dr. G. C. Low also came across one case, and Wenyon saw three others at the London Hospital.

Wenyon (21) has reported briefly but intelligibly on these three cases and confirms Woodcock's conjecture that his cases were of *Isospora* infection. Wenyon comments on these cases as follows:

As the coccidium develops in the intestinal epithelium, it of course brings about destruction of the epithelial cells themselves, and so must be regarded as of some pathogenic importance, although the symptoms of human intestinal coccidiosis have not been definitely determined. In animals, such infections are often the cause of serious enteritis, which may have a fatal termination.

Wenyon continued his study of this parasite (22) and figures the oöcysts in various stages of development. The cysts are oval, measuring  $27\ \mu$  to  $30\ \mu$  by  $12\ \mu$  to  $15\ \mu$ , and contain two sporocysts, each containing four sporozoites and a mass of residual protoplasm.

In still another paper (20) this same author reports a case of infection with *Coccidium* in a soldier invalided home from Gallipoli. This case is interesting in that the cysts passed by this patient did not in the least resemble the cyst of the rabbit *Coccidium*, but more closely resembled the cysts of *Coccidium falciforme* found in the intestine of the mouse. They were almost spherical, measuring  $20\ \mu$  in diameter, whereas *Coccidium cuniculi* of the rabbit produces oval cysts that measure  $28\ \mu$  to  $42\ \mu$  by  $14\ \mu$  to  $28\ \mu$ . The oöcyst of this coccidium, as is to be expected, contains four sporocysts, each containing two sporozoites and a mass of residual protoplasm. In addition to this, the oöcyst of Wenyon's *Coccidium* was not smooth externally like that of *Coccidium cuniculi*, but was covered with irregularities in the form of small nodular ridges and elevations, and the same condition was seen in the sporocysts. Wenyon states that while his *Coccidium* resembles most nearly that of the mouse, it is impossible for him to state definitely if it is actually this species or one quite distinct.

Briefly discussing the matter of the infection of man with both *Isospora* and *Coccidium*, Wenyon says the question is one of great interest. Infection, he says, undoubtedly takes place by way of the mouth, but whether the dust, food, or water, or all three of these are involved remains to be determined. He



adds that the possibility of infection through association with animals that are passing the oöcysts in their fæces must be investigated.

The belief is current that the cysts of coccidia are exceedingly resistant to untoward environmental conditions. Apparently they are much more resistant than the cysts of species of *Entamæba*. It must, however, be said that their impermeability makes it exceedingly difficult to determine whether they are living or not, by the application of the eosin test, which gives such excellent results in the case of *Entamæba*. The department of medical zoölogy is at present conducting a series of tests to determine how long they will retain their vitality under approximately normal and under experimental conditions. This is an investigation that will consume considerable time—several years in fact, but our preliminary investigations show us that they are extraordinarily resistant to a variety of reagents that quickly kill the cysts of *Entamæba*. I have watched, under the microscope, the development of cysts of *Isospora bigemina* in a 3 per cent solution of potassium bichromate, in water treated with thymol, and in double-strength Gram's iodine solution, and I have even seen cysts that would resist the application of Bouin's picro-aceto-formol solution for more than four hours. Other workers have reported on the resistance of coccidial cysts to desiccation, but it is too early to report anything on this from our laboratory.

It would not be surprising to discover that these cysts remain viable after two or even three or more years. Indeed the cysts of *Coccidium avium* have been shown to be infective two years after passage from the intestine of the infected fowl.<sup>(7)</sup> Mast<sup>(14)</sup> has shown that the cysts of *Didinium nasutum*, a free-living infusorian, will retain their vitality for a period of five years in air-tight vials. He has found that drying in ordinary atmospheric conditions does not destroy the cysts. In fact, he believes they will live longer dry than in a solution.

However, Wenyon and O'Connor<sup>(24)</sup> corroborate Kuenen and Swellengrebel in their assertion that the cysts of *Entamæba histolytica* will not withstand drying. They add that cysts of this parasite will survive for thirty days in water, again confirming Kuenen and Swellengrebel. They point out the importance, however, of making a considerable dilution with water to keep down bacterial and fungoid overgrowths that tend to destroy the cysts.

This is in keeping with the findings of Hadley,<sup>(9)</sup> who advises the study of cyst development in *Coccidium* in 5 per cent

solutions of potassium bichromate, which will arrest putrefactive changes that tend to bring about abnormal development of the cysts.

From the foregoing it will be seen that the disposal of matter containing coccidial cysts may prove to be a very troublesome problem. Quicklime seems to exert the most destructive action on these cysts of any of the disinfectants in common use.

Cysts of species of the genus *Coccidium* are frequently passed in a stage of advanced development, so that they may become infective very soon after they leave the intestinal tract of the original host. But development to the sporozoite stage in the case of *Isospora* does not, as a rule, become complete until two or three days have elapsed after the cysts have left the host. It should be understood that the sporozoites are the end product of sporogony, and they are the form in which the parasite enters the epithelium of the new host. If a cyst in a developmental stage preceding sporozoite formation is taken into the alimentary tract of a new host, the cyst envelope is dissolved and an inert mass of protoplasm is liberated that is incapable of doing harm and that probably very quickly disintegrates.

Notwithstanding the immense amount of work that has been done on this group of the Protozoa, apparently very little has been done on the specificity of these parasites to particular hosts. *Coccidium cuniculi* is generally credited with being infective to rabbits, dogs, cattle, and man, it being suggested that man may become infected through eating livers of rabbits containing the sporocysts of the coccidium. This theory seems to me to be untenable. In the first place the cysts seem to be unable to complete their development in the liver and in many cases eventually degenerate. Furthermore the presence of carbon dioxide in the liver apparently exerts an exceedingly deleterious effect upon them, resulting finally in their destruction.<sup>(1)</sup> Such a condition is frequently found in old rabbits that have spontaneously recovered from an earlier coccidial infection. But even allowing that these causes may not always be operative, it seems to me hardly likely that the cysts will retain their vitality through the process of cooking, even though they be walled off from the general mass of the liver by connective tissue capsules. A method of infection similar to that obtaining in the case of *Entamoeba histolytica* or other protozoa or helminths of similar habitat seems to me much more likely.

On the other hand, on contrasting the two almost similar species, *Coccidium avium* of birds and *Coccidium cuniculi* of the rabbit, it is seen that the rabbit coccidium will not infect birds

and conversely that the bird coccidium will not infect rabbits.

*Isospora bigemina* is known to be infective to dogs, cats, and polecats, and is suspected of being infective to man, although Wenyon and O'Connor<sup>(23)</sup> report negative results on feeding a kitten and a mouse the developed cysts of *Isospora* taken from a human case in Egypt. They add that *Isospora* is frequently found in cats in Alexandria, but that the oöcyst "is quite unlike that of the human parasite. The oöcysts of the cat isospora resemble those of the European form."

However, the size of the oöcysts is not a safe criterion on which to found species any more than is the number of merozoites formed by a schizont in the asexual phase. These variations are found within the species and may be almost as striking as the variations found in the size of the trypanosomes. They are governed largely by conditions within the host—particularly in regard to the food supply. Multiple infections of epithelial cells modify the parasite greatly, and these modifications appear in the asexual phase as reductions in the size of trophozoites and in the number of merozoites produced and in the sexual phase in the production of smaller cysts, this being rather strikingly illustrated by the *Coccidium* found here by Dr. Elias Domingo. In the strain of *Isospora bigemina* carried on in our laboratories the cysts have been uniform in size, no very striking differences having so far appeared.

All of this probably accounts in a large degree for the lack of uniformity in size of the cysts of known species as reported by different observers and points out the need for added information on the problems of cross-infectivity and the dangers these animal species hold for human beings.



FIG. 2. Completely developed cyst of *Coccidium cuniculi*.

Regarding the coccidia on which we are at present working, little can be said at this time, so I shall direct your attention to the illustrations that accompany this article. The cysts of Doctor Domingo's *Coccidium* tend toward the oval in shape, though some are nearly spherical. The oval cysts measure about  $20\ \mu$  by  $16\ \mu$ , while those that are spherical measure from  $19\ \mu$  to  $20\ \mu$  in diameter, which brings them rather close, in point of size, to the measurements recorded by Wenyon for his human coccidium.

The cysts of *Coccidium cuniculi* range from a



FIG. 1. Cyst of *Isospora cuniculi* in early stage of development.



length of  $33\ \mu$  to  $49\ \mu$ , with a breadth of from  $15\ \mu$  to  $28\ \mu$ . There are few, if any, data regarding the size of *Coccidium cuniculi* cysts recovered from human cases.

Measurements of a few cysts of our cat *Isospora* show a variation in length of from  $29\ \mu$  to  $38\ \mu$  and in breadth of from  $22\ \mu$  to  $29\ \mu$ , all cysts being oval. These figures do not wholly coincide with measurements of cysts, presumably of the same species, made by other workers. Stiles(18) reports measurements of from  $24\ \mu$  to  $40\ \mu$  in length and from  $19\ \mu$  to  $28\ \mu$  in breadth, while Swellengrebel(19) gives a length of from  $39\ \mu$  to  $47\ \mu$  and a breadth of from  $26\ \mu$  to  $37\ \mu$ .

Wenyon apparently had no opportunity to measure large numbers of the cysts of his human *Isospora*, and measurements taken from the scale on his figures give a length of from  $27\ \mu$  to  $30\ \mu$  and a breadth of from  $12\ \mu$  to  $15\ \mu$ . The coccidium of Railliet and Lucet,(16) which Fantham(8) seems inclined to place in the genus *Isospora*, was reported by these writers to form cysts measuring  $15\ \mu$  by  $10\ \mu$ . Lastly Doctor Domingo's *Isospora* of the lizard seems to measure from  $16.5\ \mu$  to  $27.6\ \mu$  in length by from  $14\ \mu$  to  $23\ \mu$  in breadth.

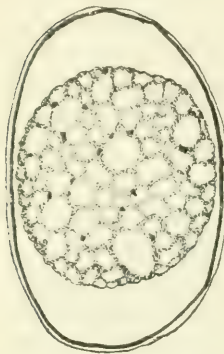


FIG 3. Cyst of *Isospora bigemina* in an early stage of development.

Other figures might be quoted, but it seems to me that they offer us little help in our problem of determining the pathogenicity of the coccidia of the lower animals to man.

And now in conclusion let me say a word about the identification of these cysts. It is probably needless for me to say that this department would welcome the opportunity to work up any material of this nature that may be sent to it. The main thing that the observer must be on his guard against is the confusing of the cysts of helminths, particularly the eggs of trematodes and of hookworms, with coccidial cysts and vice versa. As a rule, the eggs of helminths will be found to be much larger than the sporozoan cysts, but there is no denying the fact that unsegmented eggs of this type do bear a striking resemblance to the oöcysts of coccidia, and this resemblance may even extend to the early stages of segmentation of the eggs, particularly the two-cell stage. The best plan is to dilute the stool with a considerable quantity of water and set a number of the cysts aside in a moist chamber for two or three days.

In the coccidial cysts the protoplasm entirely fills the cyst

in the early stage, but later it contracts to a spherical mass in the center, leaving clear spaces at each pole of the cyst. Eventually this mass divides, in the case of *Isospora* into two masses and in the case of *Coccidium* into four masses. A cyst membrane forms around each of these daughter masses, and some hours later sporozoites and a mass of residual protoplasm can be made out in each of the sporocysts that are contained within the oöcyst. *Isospora* forms four sporozoites in each sporocyst and *Coccidium* two. This establishes the identification beyond a doubt. All these changes may be seen by making examinations of the cysts under the microscope at varying intervals, or the cysts may simply be set aside in the moist chamber for from forty-eight to seventy-two hours and then examined. It is a comparatively simple matter to isolate individual cysts with a capillary pipette under the microscope.

Finally I desire to say that while I do not predict that coccidia will be found infesting human beings in the Philippine Islands, still, in view of the fact that we have found coccidia in domestic animals here, I see no reason why human coccidiosis should not occur here as it has in other places. It is not hard to see how occasional cases may have been overlooked in the past or may be overlooked in the future. It is on the general practitioner that we must largely rely for the opportunity to develop our knowledge of this very important condition, and the need for that knowledge seems to me to be imperative.

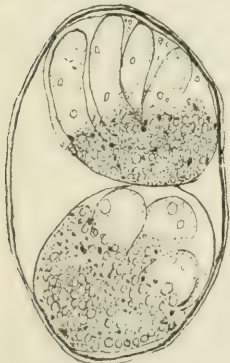


FIG. 5. Completely developed cyst of *Isospora bigemina*.

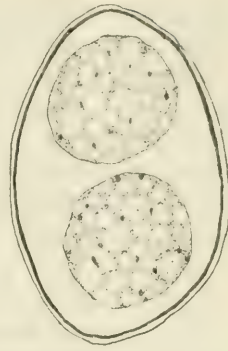


FIG. 4. Cyst of *Isospora bigemina*, showing development of sporocysts.

NOTE.—Since the above was written I have read the paper of Savage and Young,<sup>2</sup> in which they report the finding of six cases of infection with “*Coccidium isospora*.” It seems safe to assume that the writers were dealing with infections by parasites of the genus *Isospora*. Five of these cases were in the hospital under the observation of the authors. All of these five patients were suffering from dysentery. Two had the

<sup>2</sup> Report on the treatment of fifty-nine cases of *Entamoeba histolytica* infection, *Journ. Roy. Army Med. Corps* (1917), 29, 249.

acute entamœbic type, two were bacillary, and one was suffering from a chronic entamœbic infection.

Treatment with emetine compounds had no effect whatever on the sporozoan infection. One case was treated especially for the elimination of the *Isospora*, by the hypodermic administration of emetine hydrochloride over a period of nine days, thirteen grains in all being given. The infection was "practically unaffected." Then silver nitrate injections were tried. A solution of 1 in 2,000, one pint, was given for eight days. The writers report the disappearance of the "coccidia" after three days of treatment; they were found on one occasion only, four days after the last silver nitrate injection. Daily examinations were then made for sixteen days, during which the stools were free from the infection.

However, the authors go on to say that in all the other cases except one the parasites disappeared from the stools without special treatment. This, to say the least, is suggestive of confirmation of the view that the development of sporogony automatically purges the host of its infection in a large proportion of cases. It should not however, give us a false sense of security, for the possibility of liver complications should not be overlooked.

Experience with ipecac and the emetine compounds in protozoan infections other than those with *Entamœba histolytica* should lead us to expect negative results in the treatment of coccidial infections. There is very little evidence to show that either ipecac or emetine has any but the slightest effect on *Entamœba coli* or any of the flagellated protozoa. At the same time it is not certain from the report of Savage and Young that the silver nitrate injections worked a cure in the case in which they were tried. Nevertheless it seems reasonable to assume that in view of the fact that coccidial infections are, for the most part, limited to the epithelial layer of the intestine, local treatment would tend to be more effective than in the case of *Entamœba histolytica*, which works its way deep into the submucosa, where it is safe from the action of drugs similarly applied.

In still another recent article, Castellani<sup>2</sup> states that coccidiosis is comparatively common in the Balkans. He cites fourteen cases reported by Richards from the 43d General Hospital, Salonika, and six cases seen by himself in Macedonia. Of the latter group two, he says, exhibited diarrhœa, but the others

<sup>2</sup> *Journ. Trop. Med. & Hyg.* (1917), 20, 202.



showed no intestinal symptoms. He describes the treatment as having been unsatisfactory following the use of emetine and a long series of "so-called intestinal disinfectants," all of which yielded very poor results. In two cases methyl blue seemed to act beneficially.

The foregoing data yield us at least thirty-four well-authenticated cases of human coccidiosis of recent occurrence, which should act as a stimulus to future research into this condition.

## REFERENCES

- (1) BRAUN, MAX., and LUHE, M. A Handbook of Practical Parasitology. Wm. Wood & Co., New York (1915), 73.
- (2) DAVAINÉ, C. Trait des Entozoaires, etc. Ed. 2e, Paris (1877), 269.
- (3) DUTTON and TODD. Flagellata in the blood of a mouse. *Mem. Liverpool School Trop. Med.* (1903), 11, 56.
- (4) FANTHAM, H. B. Insect flagellates and the evolution of disease, with remarks on the importance of comparative methods in the study of protozoölogy. *Ann. Trop. Med. & Parasit.* (1915), 9, 335.
- (5) IDEM. Remarks on the nature and distribution of parasites observed in the stools of 1,305 dysenteric patients. *Lancet* (1916), 1, 1165.
- (6) FANTHAM, H. B., and PORTER, ANNIE. Some experimental researches on induced herpetomoniasis in birds. *Ann. Trop. Med. & Parasit.* (1915), 9, 543.
- (7) FANTHAM, STEPHENS and THEOBALD. The Animal Parasites of Man. John Bale Sons and Danielson, London (1916), 145.
- (8) IDEM. Ibid. (1916), 149.
- (9) HADLEY, PHILIP B. Eimeria avium: A morphological study. *Arch. f. Protistenk.* (1911), 23, 7.
- (10) KREMPE, A. Sur un hématozoaire endoglobulaire nouveau de l'homme (Haemogregarina hominis). *Compt. rend. Acad. sci.* (1917), 164, 965.
- (11) LANFRANCHI, A. Su la possibile trasmissione delle tripanosomiasi animali nell' uomo. *Bull. Sci. Med.* (1915), 86, Ser. 9, Vol. 3, 18 pp. Cited in *Trop. Dis. Bull.* (1916), 8, 274.
- (12) IDEM. Ulteriori ricerche sulla possibile trasmissione delle tripanosomiasi animali nell' uomo. Le reazioni biologiche nelle tripanosomiasi umana ed animali nella identificazione dei virus. Notes I, II, III. *Rendiconti d. R. Accad. d. Lincei* (1916), 25, ser. 5a, 195-198; 230-234; 601-605. Cited in *Trop. Dis. Bull.* (1916), 8, 274.
- (13) LYNCH, KENNETH M. The rat a carrier of a dysenteric ameba. *Journ. Am. Med. Assoc.* (1915), 65, 2232.
- (14) MAST, S. O. See abstracts of *Proc. Am. Soc. Zool.*, Annual Meeting for 1916, p. 62.
- (15) MINCHIN, E. A. An introduction to the study of the Protozoa. Edward Arnold, London (1912), 291.
- (16) RAILLIET and LUCET. Obs. s. quelq. Cocc. intest. *Compt. rend. Soc. biol.* (1890), 660; Railliet, Trait. Zool. med. et agric., 2e ed. (1895), 140. Cited by Fantham, Stephens and Theobald.
- (17) SMITH, THEOBALD. Aberrant intestinal protozoan parasites in the turkey. *Journ. Exp. Med.* (1916), 23, 293.

- (18) STILES, CH. W. Notes on parasites. *Journ. Comp. Med. & Vet. Sci.* (1892), 13, 517; *Bull. Soc. zool. France* (1891), 16, 163.
- (19) SWELLENGREBEL, N. H. Zur Kenntnis der Entwicklungsgeschichte von *Isospora bigemina* (Stiles). *Arch. f. Protistenk.* (1913), 32, 379.
- (20) WENYON, C. M. Another human coccidium from the Mediterranean war area. *Lancet* (1915), 2, 1404.
- (21) IDEM. The common intestinal protozoa of man. *Ibid.* (1915), 2, 1173.
- (22) IDEM. The development of the oöcyst of the human coccidium: An addendum. *Ibid.* (1915), 2, 1296.
- (23) WENYON, C. M., and O'CONNOR, F. W. An inquiry into some problems affecting the spread and incidence of intestinal protozoal infections of British troops and natives in Egypt, etc. *Journ. Roy. Army Med. Corps* (1917), 28, 686.
- (24) IDEM. The carriage of cysts of *Entamoeba histolytica* and other intestinal protozoa and eggs of parasitic worms by houseflies with some notes on the resistance of cysts to disinfectants and other agents. *Ibid.* (1917), 28, 522.
- (25) WOODCOCK, H. M. *Brit. Med. Journ.*, Nov. 13, 1915. Cited by Wenyon (No. 21).

## ILLUSTRATIONS

### TEXT FIGURES

- FIG. 1. Cyst of *Coccidium cuniculi* in early stage of development.  
2. Completely developed cyst of *Coccidium cuniculi*.  
3. Cyst of *Isospora bigemina* in an early stage of development.  
4. Cyst of *Isospora bigemina*, showing development of sporocysts.  
5. Completely developed cyst of *Isospora bigemina*.





## EXPERIMENTS ON THE TREATMENT OF RINDERPEST WITH VARIOUS DRUGS <sup>1</sup>

BY WILLIAM HUTCHINS BOYNTON

(*From the Bureau of Agriculture, Manila*)

The following experiments have been accomplished at various times during a period of approximately six and one-half years. The veterinary division, of the Bureau of Agriculture, is frequently confronted with men who claim to have specific cures for rinderpest, and when their so-called cures are given the proper trial, they are found lacking in curative powers. On account of these frequent claims and rumors of cures it was thought advisable at this time to publish the results of our experiments on various drugs, as these results may aid in obtaining a better idea of a method of treatment and may also give a clearer insight into the location of the fountain head of the virus in the animal body.

In the laboratory only highly susceptible animals are used, which are obtained from localities where rinderpest has supposedly never existed or where a considerable number of years has elapsed since the last appearance of the disease. A highly virulent strain of virus is also used in all the work. The virulence of the strain is kept up by taking the infective material from animals in the early stage of the disease, that is, the first or second day after the initial rise in the temperature. If the infective material is obtained from animals in the last stages of the disease, the virus soon loses its potency, undoubtedly due to the action of the antibodies upon it, which have a tendency to weaken the virus and to render the results unreliable.

If the records of the veterinary division, Bureau of Agriculture, are consulted, it will be notice that in those localities where the cures for rinderpest have been so successful the recovery of animals under normal conditions has been very high, averaging in many instances in the neighborhood of 60 per cent. If the person administering the cure is at all shrewd, he can easily eliminate the doubtful cases and in that way obtain a high percentage of recoveries from his drugs, providing the drugs are not too harmful to the animals and are administered in small enough doses.

<sup>1</sup> Published in *Phil. Agr. Rev.* (1917), 10, 272.

The drugs used in the following experiments were as follows: (1) Eosin; (2) medicinal methylene blue (Merck); (3) cacodylate of soda; (4) atoxyl; (5) quinine sulphate; (6) camphorated oil; (7) creolin; (8) permanganate of potash; (9) ergot; (10) iodine; (11) potassium iodide; (12) gentian violet; (13) adrenalin hydrochloride; (14) nuclein; (15) formalin; (16) chlorazene; (17) castor oil; (18) alcohol; (19) fluid extract of *nux vomica*; and (20) fluid extract of gentian.

With the small amount of experimentation that has been given to each drug, no promising results have been obtained by the method in which they were administered and the dosage in which they were given.

In all the experiments where sodium chloride solution was made use of to dilute the drug for intravenous and intraperitoneal injections, 0.85 per cent was used. It was found when giving large intravenous and intraperitoneal injections that if the fluid was warmed to about 41° C. the animals withstood the injections with much less discomfort than when the solutions were cooler. All the large intravenous injections were performed in the manner illustrated in Plate I.

#### EOSIN

Eosin was used with the idea that it might have a special affinity for the virus of rinderpest, at it is one of the diffuse stains and penetrates well.

#### EXPERIMENT 1

Batanes bull 3153, which had contracted rinderpest by exposure to sick animals and had run a high temperature for forty-eight hours, was injected subcutaneously on the afternoon of May 30, 1911, with 2 grams of Grubler's W. Gelb eosin dissolved in 100 cubic centimeters of sterile distilled water.

July 1 this animal's temperature subsided to normal, but it developed inappetence and diarrhoea and died July 3, presenting good lesions of rinderpest upon autopsy. The subcutaneous and mesentery tissues had taken on a pinkish coloration, resulting from the free distribution of eosin throughout the body, which apparently had no ill effects upon the virus of rinderpest.

#### MEDICINAL METHYLENE BLUE

Methylene blue was used in experiments 2 and 47 with the idea that if perchance the virus of rinderpest was an intracorporeal organism this dye might have a direct action upon it, as is the case in malaria. Its antipyretic and anodyne actions were also considered.



## EXPERIMENT 2

Batanes bull 3116 was injected on May 23, 1911, with 30 cubic centimeters of filtered blood handled as follows: Five cubic centimeters of virulent rinderpest blood from bull 3135 was diluted up to 500 cubic centimeters with sterile 0.85 per cent sodium chloride solution; this diluted blood was then passed through a Berkefeld N. filter under 3 kilograms' pressure.

This animal presented its first rise in temperature on the morning of May 28, registering 39.2° C.

During the afternoon of May 29, or thirty-six hours after the first rise in temperature, this animal was injected subcutaneously with 2 grams of Merck's medicinal methylene blue dissolved in 100 cubic centimeters of sterile distilled water.

May 30 this animal's urine was dark blue. Its visible mucous membranes also took on a bluish coloration.

May 31, diarrhoea, eating little.

June 1-3, diarrhoea, not eating.

June 4, died, presenting good lesions of rinderpest upon autopsy.

The methylene blue thus administered had no apparent ill effect upon the virus of rinderpest.

## CACODYLATE OF SODA

Cacodelate of soda was tried with the idea that it might have an action upon the virus of rinderpest similar to that which it has upon *Treponema pallidum*, although arsenobenzol (salvarsan, 606) has been tried on rinderpest with negative results. (The reference to the experiments with salvarsan cannot be located.)

## EXPERIMENT 3

Carabao 3088 had contracted rinderpest by exposure to sick animals.

May 17, 1911, the fourth day of temperature, this animal was injected intravenously in the femoral vein with 6 grains of cacodylate of soda. The animal's temperature subsided to normal in one and one-half days after the injection, but it suffered severely from photophobia; diarrhoea, not eating.

The animal died the night of May 19, presenting marked lesions of rinderpest.

## EXPERIMENT 4

*Batanes bull 3158.*—This animal had been injected with virulent rinderpest blood.

May 19, 1911, which was the second day of temperature, this animal was injected intravenously in the femoral vein with 5.25

grains of cacodylate of soda. The temperature decreased on May 20, but the animal presented marked symptoms of photophobia.

May 21-23, diarrhœa, not eating.

This animal died May 23, presenting marked lesions of rinderpest upon autopsy.

#### EXPERIMENT 5

*Batanes bull 3135.*—This animal had been injected with virulent rinderpest blood.

May 23, 1911, eighteen hours after the first rise in temperature, this animal was injected intravenously in the femoral vein with 3 grains of cacodylate of soda.

May 24, this animal presented symptoms of photophobia.

May 25, eating little.

May 26-28, diarrhœa, not eating.

May 28, died of rinderpest, presenting marked lesions.

From the results of experiments 3, 4, and 5, it will be seen that cacodylate of soda as it was used in these cases had no ill effect upon the virus of rinderpest and, if anything, aggravated the disease, causing a much more pronounced photophobia than is normally present in rinderpest and also more pronounced intestinal lesions than are usually noticed. From these results it appears that arsenic compounds are contraindicated in rinderpest.

#### ATOXYL

Atoxyl was tried because it has such a pronounced action in clearing the blood stream of trypanosomes in cases of surra infection, and there was a possibility that it might have a similar action upon the virus of rinderpest.

#### EXPERIMENT 6

*Batanes bull 3119.*—This animal had been injected with 30 cubic centimeters of virulent rinderpest blood serum<sup>2</sup> on July 8, 1911.

July 11, it presented a rise in temperature, registering, in the afternoon, 40.2° C.

July 12, it developed a diarrhœa.

July 14, which was the third day after the initial rise in temperature, this animal was injected subcutaneously with 5 grams of atoxyl dissolved in 60 cubic centimeters of sterile distilled water.

<sup>2</sup> Virulent rinderpest blood was allowed to stand in the ice box for twenty-four hours, then the serum was drawn off and injected.

The animal's temperature dropped to slightly high normal shortly after the injection, registering  $39.4^{\circ}$  C., but it presented marked symptoms of photophobia.

July 16, this animal died, presenting marked lesions of rinderpest upon autopsy.

The atoxyl gave symptoms similar to those of the cacodylate of soda and appeared to stimulate the virus instead of retarding its action.

#### QUININE SULPHATE

Quinine sulphate was used with the idea that it might have an action upon the virus of rinderpest similar to that which it has upon malaria. Its antipyretic action was also taken into consideration.

#### EXPERIMENT 7

*Batanes bull 4315.*—This animal was injected on November 24, 1911, with 25 cubic centimeters of rinderpest blood that had been kept in a clotted form in a large test tube for ninety-six hours in the incubator at  $37^{\circ}$  C. The clotted blood was taken from the test tube and macerated in a sterile mortar with a 5 per cent potassium citrate solution, and the liquid material thus obtained was injected.

November 30, in the forenoon, this animal presented its first rise in temperature, registering  $39.6^{\circ}$  C.

December 1–2, diarrhœa, eating little.

December 3–4, diarrhœa, not eating.

December 4, this animal was given quinine sulphate in capsules per orum in the following doses:

	Grams.
8.30 a. m.	5
11.30 a. m.	5
2.30 p. m.	5
5.30 p. m.	5

This animal died during the night of December 4 and presented good lesions of rinderpest upon autopsy.

From this experiment it will be noted that the animal received 20 grams of quinine sulphate per os with no effect upon the disease, although it was in the last stages of the disease when the treatment was undertaken.

#### EXPERIMENT 8. QUININE SULPHATE AND ERGOT

*Fuga carabao 67.*—This animal was injected on January 28, 1917, with 50 cubic centimeters of virulent rinderpest blood from carabao 65.



January 29 this animal received intraperitoneally 8 grams of quinine sulphate dissolved in 1,000 cubic centimeters of sterile distilled water slightly acidulated with sulphuric acid. The injection was made at this time to try to abort the disease. In two hours after this injection the carabao was lying down, eating little, and presenting slight nervous symptoms.

January 30, this animal was standing up and eating well, but had a forenoon temperature of  $38.9^{\circ}\text{C}$ . and an afternoon temperature of  $39.5^{\circ}\text{C}$ .

January 31, afternoon temperature  $40.5^{\circ}\text{C}$ . The animal looked bright and was eating well.

February 1, forenoon temperature  $39.9^{\circ}\text{C}$ .; administered intraperitoneally 10 grams of quinine sulphate dissolved in 1,000 cubic centimeters of acidulated 0.85 per cent sodium chloride solution; afternoon temperature  $40^{\circ}\text{C}$ .; not eating; carabao standing up.

February 2, forenoon temperature  $39^{\circ}\text{C}$ .; diarrhoea starting; slight discharge from eyes; administered subcutaneously 8 cubic centimeters of fluid extract of ergot; afternoon temperature  $39.8^{\circ}\text{C}$ .; diarrhoea, not eating.

February 3, forenoon temperature  $39.7^{\circ}\text{C}$ .; animal very sick; sunken eyes; arched back; drooling; grinding teeth; swallowing frequently; diarrhoea, not eating; blood-stained slugs of mucus in faeces; administered 10 cubic centimeters of fluid extract of ergot subcutaneously.

February 4, carabao 67 found dead in the morning; autopsy presented good lesions of rinderpest.

#### EXPERIMENT 9. QUININE SULPHATE AND IODINE

*Batanes cow 4168*.—This animal was injected January 19, 1917, with 50 cubic centimeters of virulent rinderpest blood from bull 4164.

January 22, this animal presented its first rise in temperature, registering, in the forenoon,  $39.7^{\circ}\text{C}$ .; administered intraperitoneally 2 grams of quinine sulphate dissolved in 500 cubic centimeters of acidulated sodium chloride solution and intravenously with 1,000 cubic centimeters of sodium chloride solution in which 0.75 gram of iodine and 2 grams of potassium iodide had been dissolved; afternoon temperature  $39.7^{\circ}\text{C}$ .; muzzle moist; urine dark.

January 23, forenoon temperature  $40^{\circ}\text{C}$ .; animal looked bright; eating; faeces slightly coated with mucus containing a few flakes of blood; administered intraperitoneally 3 grams of quinine sulphate dissolved in 500 cubic centimeters of acidulated 0.85 per cent sodium chloride solution; afternoon temperature

40.5° C.; animal active; muzzle moist; nostrils looked normal; eating little.

January 24, forenoon temperature 39.5° C.; no diarrhœa; looked bright; eating little; administered intraperitoneally 3 grams of quinine sulphate dissolved in 500 cubic centimeters of acidulated sodium chloride solution.

Afternoon temperature 40° C.; not eating.

January 25, forenoon temperature 39° C.; slight diarrhœa; not eating; swallowing frequently; grinding teeth; looked fairly bright; administered intraperitoneally 3 grams of quinine sulphate dissolved in 500 cubic centimeters of acidulated sodium chloride solution.

Afternoon temperature 39.6° C.; diarrhœa, not eating.

January 26, cow 4168 found dead in the morning; autopsy presented marked lesions of rinderpest; intestinal hemorrhage very pronounced.

#### EXPERIMENT 10. QUININE SULPHATE, IODINE, AND ERGOT

*Batanes cow 4172.*—This animal was injected January 22, 1917, with 50 cubic centimeters of virulent rinderpest blood from No. 4186.

January 25, this animal presented its first rise in temperature, registering in the afternoon 40.3° C.

January 26, forenoon temperature 39.3° C.; diarrhœa, not eating; administered intraperitoneally 5 grams of quinine sulphate dissolved in 500 cubic centimeters of acidulated sodium chloride solution. It was also injected intravenously with 1,000 cubic centimeters of sodium chloride in which had been dissolved 1.5 grams of iodine and 4 grams of potassium iodide. The animal withstood the injection well. The afternoon temperature was 38.4° C., which was the average normal temperature for the healthy animals this day.

January 27, forenoon temperature 38.2° C.; diarrhœa, not eating; animal did not possess good coördination of movement; muzzle moist; administered subcutaneously 8 cubic centimeters of fluid extract of ergot; afternoon temperature 38.8° C.

January 28, forenoon temperature 37.7° C.; diarrhœa, not eating; animal lying down; respiration catchy; blood in fæces; large amount of mucus; administered subcutaneously 5 cubic centimeters of fluid extract of ergot.

Afternoon temperature 38.8° C.; diarrhœa; better in appearance; breathing regular; lying down; not eating, but drinking a little.

January 29, animal found dead in the morning. Upon autopsy

the fauces presented good lesions of rinderpest. The intestinal tract did not show marked hemorrhagic lesions.

#### IODINE

Iodine was experimented with after reviewing the results of the work done by Lambert,<sup>(1)</sup> in which he proves that iodine can be used in strong enough dilution to destroy staphylococci and still have no deleterious action upon living tissue cells. It was thought that by using iodine in sufficiently large doses there might be a possibility of destroying or attenuating the virus to such an extent that the animal would be able to develop resistance enough to recover. Potassium iodide was used to facilitate the solution of iodine and also to have a direct action upon the lymphatic system, which is markedly affected in rinderpest.

#### EXPERIMENT 11. IODINE AND POTASSIUM IODIDE

*Batanes bull 4164.*—This animal was injected January 13, 1917, with 50 cubic centimeters of virulent rinderpest blood from No. 4165.

January 16, this animal presented a rise in temperature, registering, in the afternoon, 40° C.

January 17, forenoon temperature 39.7° C.; afternoon, 40.2° C.; diarrhœa.

January 18, forenoon temperature 39.5° C.; diarrhœa, not eating; administered intravenously 1,000 cubic centimeters of sodium chloride solution in which were dissolved 0.5 gram of iodine and 1 gram of potassium iodide; withstood injection well; afternoon temperature 39.8° C.

January 19, forenoon temperature 37.4° C.; diarrhœa, not eating; animal very sick; lying down; thick mucus discharge from nose; eyes sunken; grinding teeth; swallowing frequently; blood and mucus in fœces; catchy respiration.

This animal died during the morning of the 19th. Upon autopsy it presented marked intestinal lesions of rinderpest.

#### CREOLIN

Creolin was used with the idea of trying to disinfect the blood, by either killing or attenuating the virus of rinderpest to such an extent that the animal would be able to acquire enough resistance to combat the disease.

#### EXPERIMENT 12

*Davao carabao 3271.*—This animal was injected November 14, 1911, with 50 cubic centimeters of virulent rinderpest blood from No. 3225.



November 21, this animal presented its first rise in temperature, registering, in the afternoon,  $39.9^{\circ}$  C.

November 25–26, diarrhœa, not eating; congested eye; erosions in mouth; rash under tail.

November 27, diarrhœa, not eating; administered intravenously 1,000 cubic centimeters of 1.5 per cent creolin solution in 0.85 per cent sodium chloride; animal withstood the injection well.

November 28–29, diarrhœa, not eating; animal very sick.

November 30, animal found dead in the morning; typical lesions of rinderpest upon autopsy.

#### EXPERIMENT 13

*Batanes bull 3306.*—This animal was injected on November 21, 1911, with 25 cubic centimeters of virulent rinderpest blood that had been kept in a test tube at  $37^{\circ}$  C. for twenty-four hours.

November 26, this animal presented a forenoon temperature of  $39.2^{\circ}$  C.; afternoon,  $41^{\circ}$  C.

November 27, administered intravenously 1,000 cubic centimeters of a 1.5 per cent creolin solution in 0.85 per cent sodium chloride; the animal withstood the injection well.

November 29, not eating.

November 30, diarrhœa, not eating; administered intravenously 1,000 cubic centimeters of a 3 per cent creolin solution in 0.85 per cent sodium chloride; the animal withstood the injection well.

December 1–5, diarrhœa, not eating.

December 6, died during the morning, presenting typical lesions of rinderpest.

#### EXPERIMENT 14

*Davao carabao 3183.*—This animal was injected on November 29, 1911, with 50 cubic centimeters of virulent rinderpest blood from No. 3307.

December 3, this animal presented its first rise in temperature, registering, in the afternoon,  $40.2^{\circ}$  C.

December 5, diarrhœa, not eating; administered intravenously 1,000 cubic centimeters of a 3 per cent creolin solution in distilled water; also given a weak creolin solution to drink.

December 6, not eating; blood in urine.

December 7, diarrhœa, not eating.

December 8, died during the morning, presenting good lesions of rinderpest.

## EXPERIMENT 15

This experiment was made to test the infectivity of blood of an animal that had been given a 3 per cent creolin solution intravenously.

*Batanes bull 3314*.—December 6, 1911, this animal was injected with 25 cubic centimeters of blood taken from carabao 3183, twenty-four hours after this carabao had been injected intravenously with 1,000 cubic centimeters of a 3 per cent creolin solution. This injection was made to ascertain whether or not the virus had been killed in the blood by this heavy injection of creolin.

December 9, this animal presented a rise in temperature, registering, in the forenoon,  $39.2^{\circ}$  C.; in the afternoon,  $40.1^{\circ}$  C.

December 11–12, eating little.

December 13–14, diarrhœa, eating little.

December 15–21, diarrhœa, not eating.

December 22–23, bloody diarrhœa, not eating.

December 24, this animal died, presenting good lesions of rinderpest upon autopsy.

This proves that the virus was in virulent form in the blood of carabao 3183 at the time it was drawn and that the creolin had apparently no detrimental effect upon the virus.

## PERMANGANATE OF POTASH

Like creolin, permanganate of potash was tried for its antiseptic effect upon, and its possible attenuation of, the virus. Walker(3) has used permanganate of potash on animals sick with rinderpest and has obtained some favorable results. However, he was working with animals having a high natural immunity to the disease, which undoubtedly accounts for much of his success.

## EXPERIMENT 16

*Batanes bull 3307*.—This animal was injected on November 22, 1911, with virulent rinderpest blood that had been kept in a test tube in a clotted form for forty-eight hours at  $37^{\circ}$  C.

November 27, this animal presented its first rise in temperature, registering, in the forenoon,  $39.7^{\circ}$  C.; in the afternoon,  $40.6^{\circ}$  C.

November 29, diarrhœa beginning.

November 30, diarrhœa, not eating; administered subcutaneously 900 cubic centimeters of a 1–2,000 solution of permanganate of potash in physiological salt solution.

December 1–2, diarrhœa, not eating.

December 3, this animal was found dead in the morning and presented good lesions of rinderpest upon autopsy.

## EXPERIMENT 17

*Batanes bull 3309.*—This animal was injected on November 23, 1911, with 25 cubic centimeters of virulent rinderpest blood that had been kept in a test tube in a clotted form for seventy-two hours at 37.° C.

November 29, bull 3309 presented its first rise in temperature, registering, in the afternoon, 40.2° C.

December 1, eating little; administered intravenously 1,000 cubic centimeters of a 1–1,000 solution of potassium permanganate in physiological salt solution.

December 2, eating little.

December 3–4, diarrhœa, eating little.

December 5, diarrhœa, not eating.

December 6, died, presenting good lesions of rinderpest.

## EXPERIMENT 18

*Batanes bull 3315.*—This animal was injected on November 24, 1911, with 25 cubic centimeters of virulent rinderpest blood that had been kept in a test tube in a clotted form for ninety-six hours at 37° C.

November 30, bull 3315 presented its first rise in temperature, registering, in the forenoon, 39.6° C.; in the afternoon, 40.8° C.

December 1, diarrhœa, eating little.

December 2, diarrhœa, eating little; administered intravenously 1,000 cubic centimeters of a 1–500 solution of potassium permanganate in physiological salt solution.

December 3, diarrhœa, not eating.

December 4, diarrhœa, not eating; died during the afternoon, presenting good lesions of rinderpest.

## EXPERIMENT 19

*Davao carabao 3184.*—This animal was injected on November 29, 1911, with 50 cubic centimeters of virulent rinderpest blood from No. 3307.

December 4, carabao 3184 presented its first rise in temperature, registering, in the afternoon, 40.1° C.

December 5, a 1–300 solution of potassium permanganate was being administered intravenously, but the animal died during the injection.

From the four preceding experiments it will be noticed that potassium permanganate, whether administered subcutaneously



or intravenously, had apparently no detrimental effect upon the virus of rinderpest.

#### FORMALIN

Formalin was used on account of its potent antiseptic value, with the idea of destroying or attenuating the virus of rinderpest to such an extent that the animal would be able to recover.

#### EXPERIMENT 20

*Dalupiri carabao 3182*.—This animal was injected on November 29, 1911, with 50 cubic centimeters of virulent rinderpest blood from No. 3307.

December 3, this animal presented its first rise in temperature, registering, in the afternoon, 40.9° C.

December 4, administered intravenously 1,000 cubic centimeters of a 1-4,000 formalin solution in physiological salt solution.

December 6-7, not eating.

December 8, diarrhœa, not eating.

December 9, diarrhœa, eating little.

December 12, temperature normal; eating; no diarrhœa. This animal made a good recovery.

#### EXPERIMENT 21

Chinese bull 743 contracted rinderpest by exposure during shipment between Hongkong and Manila. This animal was in the last stages of rinderpest when treatment was tried. Bloody diarrhœa, not eating; catchy respiration; marked discharge from nostrils; still able to stand up.

July 14, 1917, administered intravenously 1,000 cubic centimeters of sodium chloride solution to which 5 cubic centimeters of 40 per cent formalin had been added. This animal died during the injection, about 600 cubic centimeters having been administered when death occurred.

#### EXPERIMENT 22

Chinese bull 742 contracted rinderpest by exposure during shipment between Hongkong and Manila. This animal was very sick; bloody diarrhœa; not eating, but strong.

July 14, 1917, administered intravenously 1,000 cubic centimeters of 0.85 per cent sodium chloride solution to which 1.5 cubic centimeters of 40 per cent formalin had been added; animal withstood injection well.

July 15, diarrhœa, not eating; flakes of blood in fæces; looked bright.

July 16, administered intravenously 1,000 cubic centimeters of sodium chloride solution to which were added 2.5 cubic centimeters of 40 per cent formalin; withstood injection well.

July 17-20, looked very sick; diarrhœa, not eating.

July 21, died this forenoon; typical rinderpest lesions.

#### EXPERIMENT 23

*Fuga carabao 137* contracted rinderpest by exposure to infected animals.

July 14, 1917, this animal presented its first rise in temperature, registering, in the afternoon, 39.9° C.

July 15, forenoon temperature 39.6° C.; afternoon, 40.4° C.; administered intravenously 1,000 cubic centimeters of sodium chloride solution to which 2 cubic centimeters of 40 per cent formalin had been added; withstood injection well.

July 16, diarrhœa, not eating; administered intravenously 1,000 cubic centimeters of sodium chloride solution to which 2.5 cubic centimeters of 40 per cent formalin had been added; withstood injection well.

July 17-20, diarrhœa, not eating; very sick; arched back; discharge from nostrils and eyes; blood in fæces.

July 20, died, presenting good lesions of rinderpest.

#### EXPERIMENT 24

*Jolo carabao 108*.—Contracted rinderpest by exposure to sick animals.

July 14, 1917, this animal presented its first rise in temperature, registering, in the forenoon, 39.1° C.; in the afternoon, 40.2° C.

July 15, administered intravenously 1,000 cubic centimeters of sodium chloride solution to which had been added 2 cubic centimeters of 40 per cent formalin; withstood injection well.

July 16, diarrhœa, eating little; administered intravenously 1,000 cubic centimeters of sodium chloride solution to which 2.5 cubic centimeters of 40 per cent formalin had been added; withstood injection well.

July 17-19, diarrhœa, not eating; very sick.

July 19, died, presenting good lesions of rinderpest.

#### EXPERIMENT 25

*Fuga carabao 131*.—Contracted rinderpest by exposure to sick animals.

July 14, 1917, this animal presented its first rise in temperature, registering, in the afternoon, 39.6° C.

July 16, administered intravenously 1,000 cubic centimeters of sodium chloride solution to which 2.5 cubic centimeters of 40 per cent formalin had been added.

July 17–20, diarrhœa, not eating; very sick; blood in fæces; mucopurulent discharge from nostrils and eyes.

July 20, died, presenting good lesions of rinderpest.

#### EXPERIMENT 26

*Fuga carabao 132.*—Contracted rinderpest by exposure to sick animals.

July 14, 1917, this animal presented its first rise in temperature, registering, in the afternoon, 40° C.

July 15, diarrhœa, not eating.

July 16, diarrhœa, not eating; administered intravenously 1,000 cubic centimeters of sodium chloride solution to which 2.5 cubic centimeters of 40 cent formalin had been added; withstood injection well.

July 17–18, diarrhœa, not eating; very sick.

July 18, died presenting good lesions of rinderpest.

In the seven experiments just described, it will be noted that one animal, Dalupiri carabao 3182, experiment 20, recovered from rinderpest. From the results of the six other experiments it may be granted that the administration of formalin played no part in the recovery, as this animal would undoubtedly have recovered without any treatment, as many animals do.

#### GENTIAN VIOLET

Gentian violet was used in this experiment with the idea that it might have some direct action upon the virus. Russell<sup>(2)</sup> has found in his research work that gentian violet has a direct action upon protozoa in high dilution, but has no detrimental effect upon tissue growing in vitro.

#### EXPERIMENT 27

Batanes bull 3931 was inoculated with rinderpest culture on July 12, 1915.

July 16, this animal presented its first rise in temperature.

July 19, administered intravenously 1,000 cubic centimeters of sodium chloride solution that had 2 grams of gentian violet dissolved in it; took injection well.

July 21–22, not eating.

July 22, died, presenting good lesions of rinderpest, showing that the virus was not affected by the injection.



## SERUM, NUCLEIN, AND ADRENALIN CHLORIDE

Nuclein was used to try to develop a leukocytosis in the animals and thus increase their resistance, since one of the chief symptoms in rinderpest is a leukopenia.

Adrenalin chloride was used to tone up the blood-vessel walls, since in rinderpest the virus or its product has a direct action upon the capillary walls, causing them to lose their tone and thus become markedly distended with blood, which leads to stasis, diapedesis, and exudation, the exudates coagulating and causing coagulation necrosis.

The serum was injected to give the animal a supply of antibodies and thus increase the resistance.

## EXPERIMENT 28

*Batanes bull 4165.*—This animal was inoculated on January 5, 1917, with 50 cubic centimeters of virulent rinderpest blood from bull 4162.

January 9, bull 4165 presented its first rise in temperature, registering, in the forenoon,  $39.2^{\circ}$  C.

January 10, in the forenoon, administered subcutaneously 200 cubic centimeters of antirinderpest serum and 15 cubic centimeters of nuclein solution; in the afternoon, 10 cubic centimeters of adrenalin chloride.

January 11, in the forenoon, administered subcutaneously 15 cubic centimeters of nuclein solution and 10 cubic centimeters of adrenalin chloride; in the afternoon, administered 15 cubic centimeters of nuclein solution and 10 cubic centimeters of adrenalin chloride.

January 12, in the forenoon, animal looked bright; slight diarrhœa; temperature  $38.5^{\circ}$  C.; administered subcutaneously 15 cubic centimeters of nuclein solution and 10 cubic centimeters of adrenalin chloride; in the afternoon, not looking so well; diarrhœa, not eating; administered 15 cubic centimeters of nuclein solution and 10 cubic centimeters of adrenalin chloride; temperature  $38.8^{\circ}$  C.

January 13, animal very weak; temperature  $36^{\circ}$  C.; administered 10 cubic centimeters of tincture of nux vomica and 10 cubic centimeters of 70 per cent alcohol.

Animal died in the afternoon, presenting good lesions of rinderpest.

Although the administration of nuclein and adrenalin chloride appeared to hold in check the more severe symptoms of rinderpest for some time, it has no effect upon the final termination of the disease.

## DAKIN'S CHLORAZENE

Dakin's chlorazene was used with the idea of either destroying or attenuating the virus of rinderpest to such an extent that the animal would be able to overcome the disease and recover.

Chlorazene is an ideal antiseptic to use, since it has no corrosive action. It neither precipitates nor coagulates proteins, such as blood serum. It is practically nontoxic even when injected hypodermically. It is extremely stable and is a powerful disinfectant in very high dilutions.

## EXPERIMENT 29

*Batanes bull 4322.*—This animal contracted rinderpest by exposure to sick animals.

July 15, 1917, bull 4322 presented its first rise in temperature, registering, in the forenoon, 38.9° C.

July 17, administered intravenously 1,000 cubic centimeters of sodium chloride solution in which 13.8 grains of chlorazene had been dissolved; animal took injection without a struggle.

July 18, administered intravenously 1,000 cubic centimeters of sodium chloride solution in which 5 grams of chlorazene had been dissolved; the animal began to eat immediately after injection.

July 20, administered intravenously 1,000 cubic centimeters of sodium chloride solution in which 5 grams of chlorazene had been dissolved; withstood injection well.

This animal did not develop any symptoms of rinderpest except a rather high normal temperature, and it was thought possible that a cure had been effected. It was left in immediate contact with animals in various stages of the disease.

July 29, bull 4322 again presented a high temperature, registering, in the forenoon, 39° C.; in the afternoon, 40° C.

August 1, as this animal continued to run a high temperature, it was decided to administer chlorazene again. It received intravenously 1,000 cubic centimeters of sodium chloride solution in which 4 grams of chlorazene had been dissolved.

August 3, diarrhoea, not eating; administered intravenously 1,000 cubic centimeters of sodium chloride solution in which 4 grams of chlorazene had been dissolved.

August 4, diarrhoea, not eating; very sick.

August 5, died during the night of August 4, presenting good lesions of rinderpest.

There is a question as to whether bull 4322 was suffering from rinderpest during the first administration of the drug. If it was suffering from rinderpest, the chlorazene injections evi-

dently destroyed the virus and also the few antibodies that may have been formed, as the second attack was as virulent as is noticed in any untreated animal, and the administration of chlorazene during this attack had no ill effect upon the virus.

## EXPERIMENT 30

*Fuga bull 4305.*—This animal contracted rinderpest by being inoculated with material from bull 4298.

July 17, 1917, afternoon temperature 40.3° C.

July 18, administered intravenously 1,000 cubic centimeters of sodium chloride solution in which 2 grams of chlorazene had been dissolved; animal withstood injection well.

July 19, administered intravenously 1,000 cubic centimeters of sodium chloride solution in which 5 grams of chlorazene had been dissolved.

July 20, administered intravenously 1,000 cubic centimeters of sodium chloride solution in which 5 grams of chlorazene had been dissolved.

July 21–22, not eating.

July 23, bloody diarrhœa; not eating; administered 2,000 cubic centimeters of sodium chloride solution in which 10 grams of chlorazene had been dissolved.

July 24, found dead in the morning; good lesions of rinderpest.

## EXPERIMENT 31

*Jolo carabao 96.*—This animal contracted rinderpest by exposure to sick animals.

July 18, 1917, carabao 96 presented its first rise in temperature, registering, in the forenoon, 39° C.; administered intravenously 1,000 cubic centimeters of sodium chloride solution in which 3 grams of chlorazene had been dissolved.

July 19, diarrhœa; administered intravenously 1,000 cubic centimeters of sodium chloride solution in which 5 grams of chlorazene had been dissolved.

July 20, diarrhœa, not eating; administered intravenously 2,000 cubic centimeters of sodium chloride solution in which 10 grams of chlorazene had been dissolved.

July 21, diarrhœa, not eating, sunken eyes; discharge from nostrils; arched back; lopping ears; ulcers in mouth.

July 22, diarrhœa, not eating; administered intravenously 2,000 cubic centimeters of sodium chloride solution in which 10 grams of chlorazene had been dissolved.

July 23, found dead in the morning; good lesions of rinderpest.



## EXPERIMENT 32

*Fuga carabao 129*.—This animal contracted rinderpest by exposure to sick animals.

July 18, 1917, carabao 129 presented its first rise in temperature, registering, in the afternoon, 39° C.

July 19, administered intravenously 1,000 cubic centimeters of sodium chloride solution in which 6 grams of chlorazene had been dissolved.

July 20, diarrhœa, not eating; administered intravenously 1,000 cubic centimeters of sodium chloride solution in which 5 grams of chlorazene had been dissolved.

July 21, diarrhœa, not eating; administered intravenously 2,000 cubic centimeters of sodium chloride solution in which 12 grams of chlorazene had been dissolved.

July 22, diarrhœa, not eating; very sick.

July 23, bloody diarrhœa; not eating; sunken eyes; discharge from nostrils and eyes; ulcers in mouth; swallowing frequently; catchy respiration.

July 24, animal found dead in the morning; marked lesions of rinderpest.

## EXPERIMENT 33

*Batanes' bull 4314*.—This animal contracted rinderpest by exposure to sick animals.

July 20, 1917, bull 4314 presented a forenoon temperature of 40.2° C.; administered intravenously 1,000 cubic centimeters of sodium chloride solution in which 6 grams of chlorazene had been dissolved.

July 21, administered intravenously 2,000 cubic centimeters of sodium chloride solution in which 12 grams of chlorazene had been dissolved; withstood injection well.

July 22, bloody diarrhœa; not eating.

July 23, bloody diarrhœa; not eating; administered intravenously 1,000 cubic centimeters of sodium chloride solution in which 5 grams of chlorazene had been dissolved; also intraperitoneally 1,000 cubic centimeters of sodium chloride solution in which 5 grams of chlorazene had been dissolved.

July 24, bloody diarrhœa; not eating.

July 25, diarrhœa, not eating; flakes of blood in mucus.

July 26, diarrhœa, not eating; flakes of blood in mucus; administered intravenously 1,000 cubic centimeters of sodium chloride solution in which 4 grams of chlorazene had been dissolved.

July 27, found dead in the morning; typical lesions of rinderpest.

## EXPERIMENT 34

*Fuga bull 4311.*—This animal contracted rinderpest by exposure to sick animals.

July 20, 1917, bull 4311 presented its first rise in temperature, registering, in the forenoon, 38.8° C.

July 21, diarrhœa, not eating; administered intravenously 2,000 cubic centimeters of sodium chloride solution in which 12 grams of chlorazene had been dissolved; animal withstood the injection well.

July 22, found dead in the morning; good lesions of rinderpest.

## EXPERIMENT 35

*Fuga carabao 125.*—This animal contracted rinderpest by exposure to sick animals.

July 20, 1917, carabao 125 presented its first rise in temperature, registering, in the afternoon, 40.4° C.

July 21, administered intravenously 2,000 cubic centimeters of sodium chloride solution in which 12 grams of chlorazene had been dissolved.

July 23, bloody diarrhœa; not eating; administered intravenously 1,000 cubic centimeters of sodium chloride solution in which 6 grams of chlorazene had been dissolved; also 1,000 cubic centimeters of sodium chloride solution intraperitoneally in which 6 grams of chlorazene had been dissolved.

July 24, bloody diarrhœa; not eating; animal very sick; sunken eyes; discharge from eyes and nostrils; ulcers in mouth, swallowing frequently.

July 25, diarrhœa, not eating; died during the forenoon; good lesions of rinderpest.

## EXPERIMENT 36

*Fuga bull 4310.*—This animal contracted rinderpest by contact with sick animals.

July 21, 1917, bull 4310 presented its first rise in temperature, registering, in the afternoon, 40.2° C.

July 22, administered intravenously 2,000 cubic centimeters of sodium chloride solution in which 9 grams of chlorazene had been dissolved.

July 23, administered intravenously 2,000 cubic centimeters of sodium chloride solution in which 10 grams of chlorazene had been dissolved.

July 26, diarrhœa, not eating; administered intravenously 1,000 cubic centimeters of sodium chloride solution in which 4 grams of chlorazene had been dissolved.

July 27, diarrhoea, not eating.

July 28, found dead in the morning; good lesions of rinderpest.

From the results obtained in experiments 29 to 36, inclusive, it will be noted that chlorazene by the method in which it was administered had no curative effect upon rinderpest.

#### SALICYLATE OF MERCURY

Salicylate of mercury was used with the idea that it might have a specific action upon the virus of rinderpest. By hypodermic injection rapid and powerful action is obtained, free from gastrointestinal irritation, which has to be considered when treating rinderpest.

In some of the experiments serum was used simultaneously with the salicylate of mercury to try to increase the resistance of the animals toward the disease.

#### EXPERIMENT 37

*Fuga carabao 128.*—This animal contracted rinderpest by exposure to sick animals.

July 21, 1917, carabao 128 presented its first rise in temperature, registering, in the afternoon, 39.8° C.

July 24, diarrhoea, not eating; given a deep intramuscular injection of 0.5 gram of salicylate of mercury suspended in 12.5 cubic centimeters of paraffin oil.

July 25, diarrhoea, not eating; given a deep intramuscular injection of 0.5 gram of salicylate of mercury suspended in 12.5 cubic centimeters of paraffin oil.

July 26, diarrhoea, not eating; given a deep intramuscular injection of 0.5 gram of salicylate of mercury suspended in 12.5 cubic centimeters of paraffin oil.

July 27, bloody diarrhoea; not eating; animal very sick.

July 28, found dead in the morning; good lesions of rinderpest.

#### EXPERIMENT 38

*Jolo carabao 98.*—This animal contracted rinderpest by exposure to sick animals.

July 24, 1917, carabao 98 presented its first rise in temperature, registering, in the forenoon, 38.9° C.; in the afternoon, 40° C.

July 25, given a deep intramuscular injection of 0.5 gram of salicylate of mercury suspended in 12.5 cubic centimeters of sterile paraffin oil.

July 26, given a deep intramuscular injection of 0.5 gram of salicylate of mercury suspended in 12.5 cubic centimeters of sterile paraffin oil.



July 27, in the forenoon, diarrhœa beginning; in the afternoon, diarrhœa, not eating.

July 28, diarrhœa, not eating.

July 29–31, bloody diarrhœa; not eating; animal very sick.

August 1, bloody diarrhœa; not eating; sunken eyes; discharge from eyes and nostrils; grinding teeth; swallowing frequently; ulcers in mouth; arched back; straining frequently; catchy respiration.

August 2, animal found dead in the morning; good lesions of rinderpest.

EXPERIMENT 39. SERUM AND SALICYLATE OF MERCURY

*Fuga carabao 127*.—This animal contracted rinderpest by exposure to sick animals.

July 27, 1917, carabao 127 presented its first rise in temperature, registering, in the forenoon, 39.1° C. It was given 300 cubic centimeters of antirinderpest serum subcutaneously and intramuscularly 0.5 gram of salicylate of mercury suspended in 12.5 cubic centimeters of sterile paraffin oil.

July 28, given a deep intramuscular injection of 0.5 gram of salicylate of mercury suspended in 10 cubic centimeters of sterile paraffin oil.

July 29, diarrhœa, not eating; given a deep intramuscular injection of 0.5 gram of salicylate of mercury suspended in 10 cubic centimeters of sterile paraffin oil.

July 30, diarrhœa, not eating.

July 31, bloody diarrhœa; not eating; animal very sick.

August 1, bloody diarrhœa; not eating; animal very sick.

August 2, bloody diarrhœa; not eating; died during the forenoon; good lesions of rinderpest.

EXPERIMENT 40. SERUM AND SALICYLATE OF MERCURY

*Batanes bull 4317*.—This animal contracted rinderpest by exposure to sick animals.

July 28, 1917, bull 4317 presented its first rise in temperature, registering, in the forenoon, 39.2° C.; given 300 cubic centimeters of antirinderpest serum subcutaneously and 0.5 cubic centimeter of sterile paraffin oil intramuscularly.

July 29, given deep intramuscular injection of 0.5 gram of salicylate of mercury suspended in 10 cubic centimeters of sterile paraffin oil; in the afternoon, diarrhœa, eating little.

July 30, diarrhœa, not eating.

July 31, bloody diarrhœa; not eating; animal very sick.

August 1, bloody diarrhœa; not eating; animal died during the forenoon; good lesions of rinderpest.

From the results obtained in experiments 37 and 38 it will be noted that the deep intramuscular injections of salicylate of mercury had no detrimental effect upon the virus of rinderpest. From the results obtained in experiments 39 and 40 it will be noted that the combination of antirinderpest serum and salicylate of mercury had no detrimental effect upon the virus of rinderpest.

## ANTIRINDERPEST SERUM

## EXPERIMENT 41

*Fuga carabao 135.*—This animal contracted rinderpest by exposure to sick animals.

July 30, 1917, carabao 135 presented its first rise in temperature, registering, in the forenoon,  $38.8^{\circ}$  C., and was injected subcutaneously with 300 cubic centimeters of antirinderpest serum.

July 31, not eating.

August 1, not eating.

August 2-3, diarrhœa, not eating.

August 4, diarrhœa, eating little.

August 5-6, bloody diarrhœa; not eating; animal very sick.

August 7, animal found dead in the morning; good lesions of rinderpest.

## EXPERIMENT 42

*Fuga carabao 134.*—This animal contracted rinderpest by exposure to sick animals.

August 10, 1917, carabao 134 presented its first rise in temperature, registering, in the afternoon,  $40.1^{\circ}$  C.

August 11, given subcutaneously 1,000 cubic centimeters of antirinderpest serum; in the afternoon, diarrhœa starting.

August 12, diarrhœa, not eating.

August 13-14, bloody diarrhœa; not eating; animal very sick.

August 15, animal found dead in the morning; good lesions of rinderpest.

## EXPERIMENT 43

*Fuga carabao 126.*—This animal contracted rinderpest by exposure to sick animals.

August 12, 1917, carabao 126 presented its first rise in temperature, registering, in the forenoon,  $39.4^{\circ}$  C.; given subcutaneously 1,000 cubic centimeters of antirinderpest serum.

August 14-15, diarrhœa, not eating.

August 16, diarrhœa, not eating; aborted.

August 17, bloody diarrhœa; not eating.

August 18, bloody diarrhœa; not eating.

August 19, bloody diarrhœa; not eating.

August 20, animal found dead in the morning; good lesions of rinderpest.

From the results obtained in experiments 41 to 43, inclusive, it will be noted that antirinderpest serum had no protective effect upon the final outcome of the disease when injected in as large a dosage as 1,000 cubic centimeters.

Antirinderpest serum is very effective when used before the symptoms of rinderpest make their appearance.

It is also beneficial when administered in large quantities to animals that have a high resistance or are infected with an attenuated strain of virus. When handling animals that are highly susceptible, such as are used in the laboratory in experimental work, when such animals are infected with a highly virulent strain of rinderpest, such as is used in the laboratory in carrying on experiments, and when this type of animal presents the first symptoms of the disease, then the administration of antirinderpest serum is of no benefit as a protective or curative agent.

#### CANABIS INDICA AND SERUM

*Canabis indica* was used for its antispasmodic, analgesic, and narcotic action and also to stimulate the appetite. It was thought that possibly by the action of the drug and the support it would receive from the antirinderpest serum there might be a chance of the animal developing enough resistance to overcome the disease.

#### EXPERIMENT 44

*Batanes bull 4313*.—This animal contracted rinderpest by exposure to sick animals.

July 31, 1917, bull 4313 presented its first rise in temperature, registering, in the afternoon, 40.2° C.

August 1, given subcutaneously 300 cubic centimeters of antirinderpest serum and intravenously 5 cubic centimeters of fluid extract of *Canabis indica*.

August 2, administered intravenously 5 cubic centimeters of fluid extract of *Canabis indica*.

August 3, diarrhœa, not eating; administered intravenously 8 cubic centimeters of fluid extract of *Canabis indica*.

August 4, diarrhœa, eating little; administered intravenously 10 cubic centimeters of fluid extract of *Canabis indica*.

August 5, diarrhœa, eating little; administered intravenously 10 cubic centimeters of fluid extract of *Canabis indica*.



August 6, diarrhœa, eating little; blood and mucus; administered subcutaneously 10 cubic centimeters of fluid extract of *Canabis indica* and given a drench of tannin solution.

August 7, diarrhœa, not eating; administered subcutaneously 10 cubic centimeters of fluid extract of *Canabis indica* and given a drench of tannin solution.

August 8, found dead in the morning; good rinderpest lesions.

#### EXPERIMENT 45

*Batanes bull 4320.*—This animal contracted rinderpest by exposure to sick animals.

August 1, 1917, bull 4320 presented its first rise in temperature, registering, in the afternoon, 39.6° C.

August 2, injected subcutaneously 300 cubic centimeters of antirinderpest serum and intravenously 5 cubic centimeters of fluid extract of *Canabis indica*.

August 3, diarrhœa, not eating; given intravenously 5 cubic centimeters of fluid extract of *Canabis indica*.

August 4, diarrhœa, eating little; given intravenously 10 cubic centimeters of fluid extract of *Canabis indica*.

August 5, diarrhœa, eating little; given intravenously 10 cubic centimeters of fluid extract of *Canabis indica*.

August 6, diarrhœa, eating little; given intravenously 10 cubic centimeters of fluid extract of *Canabis indica*.

August 7, bloody diarrhœa; not eating; given subcutaneously 10 cubic centimeters of fluid extract of *Canabis indica* and given a drench of tannin solution; animal very sick.

August 8, animal found dead in the morning; good lesions of rinderpest.

#### EXPERIMENT 46

*Fuga bull 4323.*—This animal contracted rinderpest by exposure to sick animals.

August 4, 1917, bull 4323 presented its first rise in temperature, registering, in the forenoon, 39.2° C.

August 5, diarrhœa; administered subcutaneously 500 cubic centimeters of antirinderpest serum and intravenously 5 cubic centimeters of fluid extract of *Canabis indica*.

August 6, diarrhœa, not eating; straining; very sick; administered intravenously 10 cubic centimeters of fluid extract of *Canabis indica* and given a drench of tannin solution.

August 7, bloody diarrhœa; not eating; straining; very sick; given intravenously 10 cubic centimeters of fluid extract of *Canabis indica*; given a drench of tannin solution.

August 8, animal found dead in the morning; good lesions of rinderpest.

From the results obtained in experiments 44 to 46, inclusive, it will be noticed that the administration of fluid extract of *Canabis indica* and antirinderpest serum had no effect upon the final outcome of the disease. The animals used in experiments 44 and 45 continued to eat for a much longer time than is usually the case in a fatal attack of rinderpest, and in this respect the *Canabis indica* helped them. They also did not develop ulcers in their mouths until the day before death.

#### MEDICINAL METHYLENE BLUE

##### EXPERIMENT 47

*Fuga bull 4324*.—This animal contracted rinderpest by exposure to sick animals.

August 8, 1917, bull 4324 presented its first rise in temperature, registering 39.9° C.

August 9, administered intravenously 800 cubic centimeters of sodium chloride solution in which 1 gram of medicinal methylene blue (Merck) had been dissolved; in the afternoon diarrhœa beginning.

August 10, bloody diarrhœa; not eating; administered intravenously 500 cubic centimeters of sodium chloride solution in which 0.5 gram of medicinal methylene blue had been dissolved.

August 11, bloody diarrhœa; not eating; animal very sick; administered intravenously 500 cubic centimeters of sodium chloride solution in which 0.8 gram of medicinal methylene blue had been dissolved.

August 12, animal found dead in the morning; good lesions of rinderpest.

From the results obtained in experiments 2 and 47 it will be noticed that medicinal methylene blue has apparently no detrimental effect upon the virus of rinderpest when administered either subcutaneously or intravenously.

#### MISCELLANEOUS EXPERIMENTS

The data on the following experiments cannot be located, but I have the results of these experiments in mind.

1. During 1913 a small Batanes bull suffering from an attack of rinderpest was injected intraperitoneally with quinine sulphate that had been dissolved in acidulated sodium chloride solution. This animal recovered from the disease. Without doubt the animal would have made a recovery without any

treatment, when the results of further experiments with quinine sulphate are considered.

2. During 1913 two Batanes bulls suffering from rinderpest were given subcutaneously injections of camphorated oil, which is frequently prescribed as a circulatory stimulant in septicæmia. Both of these animals died of rinderpest.

3. In the early part of 1914 two animals that were suffering from rinderpest were treated with fluid extract of gentian and fluid extract of nux vomica. These drugs were administered with the idea of keeping the circulation and appetite toned up. Both animals died of rinderpest, the drugs having practically no effect upon them, as both animals developed inappetence and diarrhœa and died in the usual length of time noticed in severe cases of rinderpest.

4. During the early part of 1914 a bull suffering from rinderpest was drenched with dilute alcohol administered at short intervals. This animal developed all the symptoms of rinderpest and died of that disease in the usual length of time.

5. During 1912 several animals were treated with castor oil, and all of them developed the usual symptoms and lesions of rinderpest and died of that disease.

#### FIELD NOTES

Dr. Stanton Youngberg, chief veterinarian, Bureau of Agriculture, and the several veterinarians in charge of the immunization stations in the provinces have been using strychnine, nitroglycerin, and echinacoid on animals that have a severe reaction while passing through the immunization. (Simultaneous method, receiving an infecting dose of virulent rinderpest blood and a supposedly protecting dose of antirinderpest serum on the same day or within one or two days of each other.)

These workers find that all three of the above-mentioned drugs prolong the life of animals by their stimulating effect and in many instances seem to sustain life long enough for the animal to develop sufficient antibodies to combat the disease and in this way make a recovery. They find that when using strychnine great care has to be taken in not stopping the use of the drug too suddenly, as its action is very transient and if not administered at short intervals the animal is apt to suffer a collapse and die suddenly.

With nitroglycerin and echinacoid the stimulating effect is of longer duration and collapse is not so frequently noticed.

Although these drugs are practically useless for animals that contract rinderpest in the usual way and have not previously



received a protecting dose of serum, the results indicate that they may be used on animals that have severe reactions while being immunized.

#### CONCLUSIONS

1. From the results of the 47 experiments in which drugs and serum were used in treating animals sick with rinderpest, it will be noticed that but one animal, carabao 3182, experiment 20, recovered from the disease and that animals of subsequent experiments (21 to 26, inclusive), treated similarly to carabao 3182, succumbed to the disease, which proves conclusively that carabao 3182 would have recovered as readily without any treatment.

2. From the result obtained in No. 1 of the miscellaneous experiments it is evident that this animal would have made a recovery without any treatment, when the results of experiments 7 to 10, inclusive, are considered. The animals in these experiments were treated in a similar manner, and all of them succumbed to the disease.

3. It will be noticed that over fifty animals were experimented upon with the various drugs mentioned and that but two animals recovered from the disease, which is positive proof that the drugs used and administered as they were had no curative power for an animal suffering from rinderpest.

#### REFERENCES

1. LAMBERT. The comparative resistance of bacteria and human tissue cells to certain common antiseptics. *Journ. Exp. Med.* (1916), 24, 683.
2. RUSSELL. The effect of gentian violet on protozoa and on tissues growing in vitro, with especial reference to the nucleus. *Journ. Exp. Med.* (1914), 20, 545.
3. WALKER. The treatment of rinderpest and hæmorrhagic septicæmia with permanganate of potash. *Journ. Comp. Path. & Therap.* (1912), 25, 185-202.



## PROCEEDINGS OF THE MANILA MEDICAL SOCIETY

REGULAR MONTHLY MEETING, DECEMBER 3, 1917

### MINUTES OF THE MANILA MEDICAL SOCIETY

The regular monthly meeting of the Manila Medical Society was held at the College of Medicine and Surgery on Monday evening, December 3, 1917. In the absence of the president and the secretary, the meeting was called to order by Dr. B. C. Crowell, president of the Philippine Islands Medical Association. On motion duly made and seconded, there being no objection, Doctor Crowell took the chair and appointed Dr. R. B. Gibson as secretary pro tempore.

Doctor Crowell presented the name of Dr. Emiliano M. Panis for membership in the Manila Medical Society. On motion, duly seconded and carried, Doctor Panis's application was referred to the council for final action.

There being no other business, the scientific program for the evening was taken up.

R. B. GIBSON,  
*Secretary pro tempore,*  
*Manila Medical Society.*

#### SCIENTIFIC PROGRAM

#### ON PHILIPPINE MEDICINAL PLANTS

By DR. LEON M. GUERRERO

The flora in the Philippines are rich in pharmacological products, the systematic pharmacological and chemical investigation of which deserves attention. Many plant preparations used by the natives or empirically prescribed by quacks have some therapeutic value. Among these may be mentioned *Alstonia scholaris* for malaria and which may serve perhaps also as a cardiac remedy; *Lunasia amara* employed as gastric sedative, but which has been found to contain an alkaloid producing nonspinal convulsions; the seeds of *Quisqualis indica*, known to the native Filipinos as a vermifuge; *Tylophora brevipes*, similar to *Tylophora asthmatica* of India, which contains an alkaloid *tylophorine* and which is popularly used as an emetic and also as an antidyseric, expectorant, and emmenagogue; *Tinospora*



*reticulata*, used for malaria and probably containing berberine; and several species of *strophanthus*, the lethal dose of which is not unknown to the natives.

#### SERUM THERAPY OF BACILLARY DYSENTERY

By DR. PEDRO T. LANTIN

Specific serum therapy for bacillary dysentery has until the last few years fallen into disrepute. The work of later investigators indicates that the serum therapy of bacillary dysentery is an effective means of checking the disease. The present paper gives the results of the treatment of 20 cases of bacillary dysentery treated with antidysentery serum. Of these 20 cases, 5 were treated medicinally and with intramuscular injections of serum, 1 death occurring; 6 were treated with serum intramuscularly and per rectum, with no deaths; 3 cases were treated with serum per rectum, with no deaths; and finally 3 cases were treated intravenously with serum, with no deaths. The majority of the cases were severe; the single case that died had been admitted to the hospital in a state of collapse. Administration per rectum was done with the patient in the knee-chest position, 30 to 50 cubic centimeters being given daily, preceded a half hour earlier by sodium carbonate (1.5 per cent) to cleanse the bowels and 60 cubic centimeters of starch-solution enema containing 10 drops of tincture of opium. Twenty cubic centimeters of serum were used twice a day when given intramuscularly, and 10 cubic centimeters every other day, intravenously with several hours previous administration of 1 cubic centimeter hypodermically to avoid anaphylaxis. The serum treatments were followed by a prompt reduction in the number of stools and a diminution of the fever. The per rectum administration of serum in the cases so treated brought about a marked alleviation of the local symptoms; it may be used in mild cases or in conjunction with intramuscular or intravenous injections. In view of the facts established in the literature, successful results should follow the use of bacillary dysentery serum in the Philippine Islands as evidenced in the cases reported by the writer.

#### DISCUSSION

Doctor Schöbl reviewed the types of dysentery bacilli and the methods of differentiating these and said that the antitoxic serum used was prepared according to Doerr with cultures of the Shiga-Kruse group. Doctor de la Paz discussed some phar-

macological aspects of the methods used in the per rectum administration of the serum, stating that the lessening of the number of stools, because of the morphine given, must be taken into consideration if this is to be considered one of the criteria of improvement as the result of the serum therapy. Doctor Calderon reported that he had experienced little success with antidysentery serum, but thought that the freshly prepared local product might be more effective. Doctor Albert stated that he has practically failed in treating dysentery in children with serum, but most of his cases were in a collapsed and dying condition when admitted to the hospital.

REMOTE MANIFESTATIONS OF FOCAL DENTAL INFECTION,  
WITH CASE REPORTS

By DR. R. FERNANDEZ

The writer emphasized the work of the last seven years on the importance of focal chronic dental infections to such conditions as arthritis, neuritis, gastritis, leucæmia, etc. He spoke of the local treatment of the abscess, the use of autogenous vaccines, and particularly the diagnosis by means of the Röntgen ray. The methods employed require 5 exposures by the extraoscular method and 12 by the intraoscular; the former is to be preferred. Skiagrams showing focal dental lesions and case histories were presented, the results of the treatment being in full accord with recent published observations.

DISCUSSION

Doctor Crowell pointed out that while attention to the teeth as a source of obscure infections is important, we must consider also the appendix, other parts of the intestines, the gall bladder, the stomach, deflected septum, and hypertrophied turbinates of the nose, the ears, and the genito-urinary organs. Doctor Ottofy quoted an article by Willoughby D. Miller, an American dentist in Berlin, written twenty-seven years ago, in which the significance of focal dental infections was pointed out; he reviewed the late development of this study in the United States and emphasized the necessity of prophylaxis, proper bridge work, and the needless extraction of teeth, many of which may be saved for the patient.

R. B. GIBSON,  
*Editor of the Proceedings,*  
*Manila Medical Society.*





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PRELIMINARY REPORT ON THE VIRULENCE OF CERTAIN BODY  
ORGANS IN RINDERPEST <sup>1</sup>

By WILLIAM HUTCHINS BOYNTON

*(From the Bureau of Agriculture, Manila)*

The following results were obtained while endeavoring to devise a method of securing the aggressins of rinderpest. Since the virus of rinderpest cannot at present be satisfactorily cultivated under artificial conditions, it was decided to try to extract the virus from the tissues of animals suffering from this disease. From the symptoms, lesions, and microscopical findings it is evident that the virus attacks primarily the involuntary muscles and the endothelial lining of the capillary vascular system and the parenchymatous tissue. This is prominently demonstrated in the intestinal tract and in the lymphatic system. Upon microscopical examination of sections of intestine from an animal that has died of rinderpest it will be found that the capillary vascular system in the mucosa is flimsy. The vessel walls are stretched, distorted, and lacking tone and are unable to return to their normal shape. This weakened condition of the vessel walls leads to congestion, diapedesis of red blood cells, and exudation of the blood plasma. As the plasma infiltrates the surrounding tissue, it coagulates, resulting in coagulation necrosis and the formation of fibrinous casts, which are constantly present in the colon of fatal cases. From the result obtained by the intravenous injection of various drugs and disinfectants(4) it is evident that the virus of rinderpest does not have its fountain head of development in the blood stream. The real place where the virus multiplies appears to be

<sup>1</sup> Published in *Phil. Agr. Rev.* (1917), 10, 410.

inside the tissue cells, where the disinfectants cannot penetrate, the virus in the blood stream being merely a surplus that is thrown off from these tissue cells. In following this line of reasoning, it was decided to consider certain tissues, where lesions were more or less pronounced, as cultures, and extracts were made from them.

The tissues used in the following experiments were liver, spleen, lymph glands, heart, intestines, thymus, skeletal muscle, larynx, pharynx, and the back of the tongue from animals that were either bled to death for virulent blood or that had died after a regular course of the disease.

The tissues were taken from the animal as soon after death as possible. The amount of tissue desired was weighed and then ground in a meat grinder that had been previously sterilized in the autoclave to keep external contamination to the minimum. The material thus prepared was placed in a sterilized flask, and twice as much phenol solution (the strength of which will be mentioned in each experiment) was added. Both crude phenol and the pure crystal form were used in these experiments with similar results, that is, 100 grams of liver were ground and placed in a sterile flask, and 200 cubic centimeters of a 0.5 per cent phenol solution were added to it. This material was kept in the refrigerator, which averages between 15° and 16° C., and was daily thoroughly agitated two or three times. In some experiments the material was placed in a shaking machine and agitated continuously for forty-eight hours at room temperature, which averages 26° C. in the morning and 28° C. in the afternoon, some days rising to 30° C. After forty-eight hours of agitation at room temperature the material was placed in the refrigerator for twenty-four hours and then filtered through gauze to separate the coarse material, and the filtrate was replaced in the refrigerator until used.

When the intestines were to be extracted, they were first thoroughly washed free from faecal matter, then placed in a 0.5 per cent phenol solution for from five to ten minutes, after which they were placed in a large container of boiled water, which was cooled to at least 37° C. The tissue was allowed to soak in this water for a few minutes to dilute the phenol that remained intact. By this method a greater percentage of the bacteria on the surface of the intestinal mucosa was destroyed. Following this treatment the tissue was weighed, passed through the meat grinder, and treated in a manner similar to that of the other tissues.

The animals used in these experiments were all highly sus-

ceptible. They were obtained from localities where, to our knowledge, rinderpest has never been introduced, or from localities where the presence of rinderpest has not been known for many years. These animals were brought to the laboratory and placed under observation for various lengths of time, which will be mentioned in each experiment. During the period of observation in the quarantine shed and throughout the course of the experiments their temperatures were taken twice daily and their general appearance was noted. The animals were kept in an isolation shed while under experimentation and until the first symptoms of disease appeared. Usually one or more susceptible animals were among them to check up any accidental infection that might gain entrance from sources other than by inoculation. As soon as the first symptoms of disease made their appearance, the animal thus affected was immediately transferred to the shed where the sick animals were kept.

The following abbreviation will be used in the experiments:

P. C. W., animals used by the Philip C. Whitaker antirinderpest serum plant.

#### EXPERIMENT 1

Water extract of liver, spleen, and lymph glands, 3 days old.

*Carabao 69.*—Known history prior to the experiment: Native Fuga carabao, 3 years old, received at the laboratory and placed in quarantine January 7, 1917. This animal was kept under observation for twelve days before it was used and at no time during this period did it present a high temperature or develop any symptoms of sickness.

February 6, 1917, carabao 69 was injected subcutaneously with 100 cubic centimeters of a 3-day-old water extract from the liver, spleen, and lymph glands of carabao 66, which was bled to death on the third day of temperature for virulent blood, to be used in immunization work. Fifty grams of each of these tissues were used, and 300 cubic centimeters of water were added and allowed to extract in the refrigerator. After three days of extraction the material was filtered through gauze, and 100 cubic centimeters of the filtrate were used in this experiment.

February 10, carabao 69 presented an afternoon temperature of 40.5° C.

February 11, forenoon temperature, 40.3° C.; afternoon temperature, 40.8° C.; diarrhœa, not eating.

February 12–13, diarrhœa, not eating.

February 14, died, presenting typical symptoms and lesions of rinderpest.



## EXPERIMENT 2

Water extract of liver, spleen, and lymph glands, 3 days old.

*Carabao 72.*—Known history prior to the experiment: Native Fuga carabao, 2 years old, received at the laboratory and placed in quarantine January 24, 1917. This animal was kept under observation for twelve days before it was used and at no time during this period did it present a high temperature or develop any symptoms of sickness.

February 6, 1917, carabao 72 was injected subcutaneously with 100 cubic centimeters of the same extract as was used in experiment 1.

February 9, carabao 72 presented an afternoon temperature of 40.5° C.

February 10–12, diarrhoea, not eating.

February 13, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest.

From the results of these two experiments it will be noticed that the watery extract from the organs used was very potent, the incubation period being four and three days, respectively, and the various symptoms leading up to death were prompt in making their appearance after the initial rise in temperature.

## EXPERIMENT 3

Phenol (0.5 per cent) extract of liver and lymph glands, 5 days old.

*Carabao 75.*—Known history prior to the experiment: Native Fuga carabao, 2 years old, received at the laboratory and placed in quarantine January 24, 1917. This animal was kept under observation for twenty-two days before it was used, and at no time during this period did it present a high temperature or develop any symptoms of sickness.

February 16, 1917, carabao 75 was injected subcutaneously with 200 cubic centimeters of a 4-day-old 0.5 per cent phenol extract from the liver and lymph glands of carabao 73, which was bled to death on the second day of temperature for virulent blood, to be used in immunization work.

The extract was prepared as follows:

Liver, 250 grams; 0.5 per cent phenol, 500 cubic centimeters.

Lymph glands, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

This was placed in the refrigerator, filtered through gauze on the third day, and returned to the refrigerator. The material had a sweet odor, presenting no evidence of putrefaction.

February 20, carabao 75 presented an afternoon temperature of 39.5° C.

February 21, morning temperature, 39° C.

February 22-23, diarrhœa, not eating.

February 24, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest.

#### EXPERIMENT 4

Phenol (0.5 per cent) extract of liver, spleen, and lymph glands, 5 days old.

*Carabao 81*.—Known history prior to the experiment: Native Fuga carabao, 2 years old, received at the laboratory and placed in quarantine January 24, 1917. This animal was kept under observation for thirty-four days before it was used, and at no time during this period did it present a high temperature or develop any symptoms of sickness.

February 28, 1917, carabao 81 was injected subcutaneously with 200 cubic centimeters of a 5-day-old 0.5 per cent phenol extract of the liver, spleen, and lymph glands from carabao 77, which was bled to death on the second day of temperature for virulent blood, to be used in immunization work.

The extract was prepared as follows:

Liver, 250 grams; 0.5 per cent phenol, 500 cubic centimeters.

Spleen, 175 grams; 0.5 per cent phenol, 350 cubic centimeters.

Lymph glands, 135 grams; 0.5 per cent phenol, 270 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator. When the extract was injected, it had a sweet odor, presenting no evidence of putrefaction.

March 2, carabao 81 presented a forenoon temperature of 39.5° C. and an afternoon temperature of 40° C.

March 4-7, diarrhœa, not eating.

March 8, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest.

#### EXPERIMENT 5

Phenol (0.5 per cent) extract of heart, 5 days old.

*Carabao 84*.—Known history prior to the experiment: Native Fuga carabao, 3 years and 2 months old, received at the laboratory and placed in quarantine January 24, 1917. This animal was kept under observation for fifty-six days before it was used in this experiment. On March 8, 1917, it was injected with 10 cubic centimeters of culture material in which the virus of rinderpest had been inoculated. The animal presented no ill effects from this injection.

March 22, 1917, carabao 84 was injected subcutaneously with

200 cubic centimeters of a 5-day-old 0.5 per cent phenol extract of the heart from carabao 85, which was bled to death on the fourth day of temperature for virulent blood, to be used in immunization work.

The extract was prepared as follows:

Heart, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator. When this extract was used, it had a sweet odor and presented no signs of putrefaction.

March 28, carabao 84 presented a forenoon temperature of  $39.6^{\circ}$  C. and an afternoon temperature of  $40.7^{\circ}$  C.

March 29, diarrhoea.

March 30, diarrhoea, eating little.

March 31, this animal was bled to death for virulent blood, to be used in immunization work. It presented good lesions of rinderpest.

#### EXPERIMENT 6

Phenol (0.5 per cent) extract of skeletal muscle, 5 days old.

*Carabao 88.*—Known history prior to the experiment: Jolo carabao, 8 years and 4 months old, received at the laboratory and placed in quarantine March 3, 1917. This animal was kept under observation for twenty-six days before it was used, and at no time during this period did it present a high temperature or develop any symptoms of sickness.

March 29, 1917, carabao 88 was injected subcutaneously with 200 cubic centimeters of a 5-day-old 0.5 per cent phenol extract of skeletal muscle from carabao 263 (P. C. W.), which was bled to death on the first day of temperature for virulent blood, to be used for hyperimmunization work in the production of anti-rinderpest serum.

The extract was prepared as follows:

Skeletal muscle, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

When the extract was injected, it had a sweet odor, presenting no evidence of putrefaction.

Carabao 88 did not develop any ill effects from this injection.

April 15, 1917, which was seventeen days after the muscle-extract injection, this animal was injected subcutaneously with



50 cubic centimeters of virulent blood, to test its susceptibility to rinderpest.

April 19, this animal presented an afternoon temperature of 40.7° C.

April 21–22, diarrhœa, eating little.

April 23–25, diarrhœa, not eating; blood and mucous casts in the fæces.

April 26, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest, which proves that it was highly susceptible to that disease when it was injected with the muscle extract. This result also leaves the impression that the virus of rinderpest is not harbored in the skeletal muscles or that it is easily destroyed in this tissue.

#### EXPERIMENT 7

Phenol (0.5 per cent) extract of liver, spleen, and lymph glands, 15 days old.

*Carabao 93.*—Known history prior to the experiment: Jolo carabao, 8 years and 4 months old, received at the laboratory and placed in quarantine March 3, 1917. This animal was kept under observation for fifty-two days before it was used, and at no time during this period did it present a high temperature or develop any symptoms of sickness.

April 25, 1917, carabao 93 was injected subcutaneously with 200 cubic centimeters of a 15-day-old 0.5 per cent phenol extract of liver, spleen, and lymph glands from carabao 251 (P. C. W.), which was bled to death on the second day of temperature for virulent blood to be used for hyperimmunization work in the production of antirinderpest serum.

The extract was prepared as follows:

Liver, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

Spleen, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

Lymph glands, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator. When the extract was injected, it had a sweet odor, presenting no evidence of putrefaction.

April 29, carabao 93 presented a forenoon temperature of 39.2° C. and an afternoon temperature 40.2° C.

April 30, diarrhœa; afternoon temperature, 40.9° C.

May 1, diarrhœa, eating little.

May 2–3, diarrhœa, not eating.

May 4, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest.

## EXPERIMENT 8

Phenol (0.5 per cent) extract of cæcum and of colon, 5 days old.

*Bull 4264.*—Known history prior to the experiment: Native Fuga bull, 4 years old, received at the laboratory and placed in quarantine April 12, 1917. This animal was kept under observation for three days before it was used; it did not present a high temperature or develop any symptoms of sickness during this time.

April 15, 1917, bull 4264 was injected subcutaneously with 100 cubic centimeters of a 5-day-old 0.5 per cent phenol extract of the cæcum and colon from carabao 251 (P. C. W.), mentioned in experiment 7.

The extract was prepared as follows:

Cæcum and colon, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator. When the extract was injected, it had a sweet odor, presenting no evidence of putrefaction.

April 20, bull 4264 presented an afternoon temperature of 40.9° C.

April 21, afternoon temperature, 41.1° C.

April 22–23, diarrhœa, not eating.

April 24, bled to death for virulent blood, to be used in immunization work. This animal presented typical symptoms and lesions of rinderpest.

## EXPERIMENT 9

Phenol (0.5 per cent) extract of larynx, pharynx, and base of tongue, 5 days old.

*Bull 4265.*—Known history prior to the experiment: Native Fuga bull, 4 years old, received at the laboratory and placed in quarantine April 12, 1917. This animal was kept under observation for seven days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

April 20, 1917, bull 4265 was injected subcutaneously with 100 cubic centimeters of a 5-day-old 0.5 per cent phenol extract of the larynx, pharynx, and base of tongue from carabao 240 (P. C. W.), which was bled to death on the third day of temperature for virulent blood to be used in immunization.

The extract was prepared as follows:

Larynx, 50 grams; 0.5 per cent phenol, 100 cubic centimeters.

Pharynx, 50 grams; 0.5 per cent phenol, 100 cubic centimeters.

Base of tongue, 50 grams; 0.5 per cent phenol, 100 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

When the extract was injected, it had a sweet odor, presenting no evidence of putrefaction.

Bull 4265 did not develop any ill effects from this injection.

May 7, 1917, which was seventeen days after the injection of the above-mentioned extracts, this animal was injected subcutaneously with 50 cubic centimeters of virulent blood.

May 10, bull 4265 presented its first rise in temperature, registering, in the forenoon, 39° C.; in the afternoon, 40° C..

May 11, forenoon temperature, 39.9° C.; bled to death for virulent blood, to be used in immunization work. This animal presented lesions found in the early stages of rinderpest. This proves that bull 4265 was susceptible to the disease when it received the injection of extracts. It also leads to the idea that the virus from these parts is either scarce or is easily destroyed by the above method of handling, as it will be noticed in experiment 10 that other tissues from the same animal were virulent eight days after extraction.

#### EXPERIMENT 10

Phenol (0.5 per cent) extract of liver, spleen, and lymph glands, 8 days old.

*Bull 4266.*—Known history prior to the experiment: Native Fuga bull, 3 years old, received at the laboratory and placed in quarantine April 12, 1917. This animal was kept under observation for ten days before it was used, and it did not present a high temperature or develop any symptoms of sickness during this period.

April 23, 1917, bull 4266 was injected subcutaneously with 50 cubic centimeters of an 8-day-old 0.5 per cent phenol extract of the liver, spleen, and lymph glands from carabao 240 (P. C. W.), mentioned in experiment 9.

The extract was prepared as follows:

Liver, 150 grams; 0.5 per cent phenol, 300 cubic centimeters.

Spleen, 150 grams; 0.5 per cent phenol, 300 cubic centimeters.

Lymph glands, 100 grams; 0.5 per cent phenol, 200 cubic centimeters.

This was placed in the refrigerator for three days; it was



then filtered through gauze, and the filtrate was returned to the refrigerator.

When the extract was injected, it had a sweet odor, presenting no evidence of putrefaction.

April 26, bull 4266 presented its first rise in temperature, registering, in the forenoon,  $38.9^{\circ}$  C.; in the afternoon,  $40.5^{\circ}$  C.

April 28–30 diarrhoea, not eating.

May 1–2, diarrhoea, not eating.

May 3, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest.

#### EXPERIMENT 11

Phenol (0.5 per cent) extract of liver, spleen, and lymph glands, 15 days old.

Three animals were used in this experiment, bulls 4269, 4270, and 4271. All these animals received a similar amount of the same extract on the same day, and all gave similar results. Therefore one only, bull 4269, will be considered.

*Bull 4269.*—Known history prior to the experiment: Native Fuga bull, 3 years old, received at the laboratory and placed in quarantine April 12, 1917. This animal was kept under observation for twelve days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

April 25, 1917, bull 4269 was injected subcutaneously with 200 cubic centimeters of a 15-day-old 0.5 per cent phenol extract of the liver, spleen, and lymph glands from carabao 251 (P. C. W.), which was bled to death on the second day of temperature for virulent blood, to be used in immunization work.

The extract was prepared as follows:

Liver, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

Spleen, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

Lymph glands, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator. The liver extract had a slight butyric acid odor when it was injected; the others were sweet.

April 27, bull 4269 presented its first rise in temperature, registering, in the forenoon,  $39.1^{\circ}$  C.; in the afternoon,  $40.3^{\circ}$  C.

April 28, forenoon temperature,  $40^{\circ}$  C.; afternoon temperature,  $41.2^{\circ}$  C.

April 30, diarrhoea, eating little.

May 1, forenoon temperature,  $40.5^{\circ}$  C.; bled to death for virulent blood, to be used in immunization work. This ani-

mal presented typical symptoms and lesions of a severe case of rinderpest.

Bulls 4270 and 4271 died of rinderpest during the forenoon of May 4. Both animals presented typical symptoms and lesions of rinderpest.

#### EXPERIMENT 12

Phenol (0.5 per cent) extract of liver, spleen, and parotid and lymph glands, 16 days old.

*Cow 4260.*—Known history prior to the experiment: Native Fuga cow, 2 years old, received at the laboratory and placed in quarantine April 12, 1917. This animal was kept under observation for twenty-one days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

May 4, 1917, cow 4260 was injected subcutaneously with 200 cubic centimeters of a 16-day-old 0.5 per cent phenol extract of the liver, spleen, and parotid and lymph glands from carabao 228 (P. C. W.), which was bled to death on the first day of temperature for virulent blood, to be used in immunization work.

The extract was prepared as follows:

Liver, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

Spleen, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

Parotid, 100 grams; 0.5 per cent phenol, 200 cubic centimeters.

Lymph glands, 150 grams; 0.5 per cent phenol, 300 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator. When the extract was injected, it had a sweet odor, presenting no evidence of putrefaction.

May 7, cow 4260 presented its first rise in temperature, registering, in the afternoon, 40.3° C.

May 10, not eating.

May 11–12, diarrhœa, not eating.

May 13, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest.

#### EXPERIMENT 13

Phenol (0.5 per cent) extract of liver, spleen, and parotid and lymph glands, 20 days old.

*Bull 4272.*—Known history prior to the experiment: Native Fuga bull, 2 years old, received at the laboratory and placed in quarantine April 12, 1917. This animal was kept under observation twenty-seven days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

May 10, 1917, bull 4272 was injected subcutaneously with 200 cubic centimeters of a 20-day-old 0.5 per cent phenol extract of the liver, spleen, and parotid and lymph glands from carabao 228 (P. C. W.), the preparation of which is described in experiment 12. The liver extract had a slight butyric acid odor at the time of injection. The other extracts had a sweet odor and presented no evidence of putrefaction.

May 14, bull 4272 presented its first rise in temperature, registering, in the forenoon,  $39.4^{\circ}$  C.; in the afternoon,  $40.4^{\circ}$  C.

May 16-18, diarrhœa, eating little.

May 19-20, diarrhœa, not eating.

May 21, animal died early in the forenoon, presenting typical symptoms and lesions of rinderpest.

#### EXPERIMENT 14

Phenol (0.5 per cent) extract of liver, spleen, and lymph glands, 15 days old.

*Carabao 92.*—Known history prior to the experiment: Native Jolo carabao, 3 years and 6 months old, received at the laboratory and placed in quarantine March 3, 1917. This animal was kept under observation for fifty-two days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

April 25, 1917, carabao 92 was injected subcutaneously with 200 cubic centimeters of a 15-day-old 0.5 per cent phenol extract of the liver, spleen, and lymph glands from carabao 251 (P. C. W.), which was bled to death on the second day of temperature for virulent blood, to be used in immunization work.

The extract was prepared as follows:

Liver, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

Spleen, 200 grams; 0.5 per cent phenol, 400 cubic centimeters.

Lymph glands, 150 grams; 0.5 per cent phenol, 300 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

When the extract was injected, it had a sweet odor, presenting no evidence of putrefaction.

April 28, this animal presented its first rise in temperature, registering, in the forenoon,  $39.4^{\circ}$  C.; in the afternoon,  $39.6^{\circ}$  C.

May 1, diarrhœa; forenoon temperature,  $39.8^{\circ}$  C.; afternoon temperature,  $41.1^{\circ}$  C.

May 2-3, diarrhœa, not eating.

May 4, diarrhœa, eating little.



May 5, this animal's temperature was normal, and it was well on the road to recovery.

June 1, carabao 92 received 50 cubic centimeters of virulent blood, which had no ill effect upon it, proving that the animal was immune to rinderpest. In addition, this animal was constantly exposed to animals in various stages of the disease.

From this result it appears that the virus in this case had become slightly attenuated by the extraction.

#### EXPERIMENT 15

Phenol (0.5 per cent) extract of liver, spleen, and lymph glands, 29 days old.

*Carabao 104.*—Known history prior to the experiment: Native Jolo carabao, 7 years and 5 months old, received at the laboratory and placed in quarantine March 3, 1917. This animal was kept under observation for seventy-one days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

May 14, 1917, carabao 104 was injected subcutaneously with 200 cubic centimeters of a 29-day-old 0.5 per cent phenol extract of the liver, spleen, and lymph glands from carabao 240 (P. C. W.), which was bled to death on the third day of temperature for virulent blood, to be used in immunization work.

The extract was prepared as follows:

Liver, 300 grams; 0.5 per cent phenol, 600 cubic centimeters.

Spleen, 300 grams; 0.5 per cent phenol, 600 cubic centimeters.

Lymph glands, 150 grams; 0.5 per cent phenol, 300 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

When the extract was injected, the liver had a slight butyric acid odor. The other extracts were sweet, presenting no evidence of putrefaction.

May 24, which was ten days after the injection, carabao 104 presented a forenoon temperature of 39° C.; afternoon, 39° C.

May 25, forenoon temperature, 39.4° C.; afternoon, 41.3° C.

May 26, diarrhœa, eating little; forenoon temperature, 39.9° C.; afternoon, 38.7° C.

May 27, the animal was eating well and its temperature registered normal. This animal rapidly recovered from the slight attack and to date has not presented any signs of rinderpest, although constantly exposed to the disease.

From the results of this experiment it appears that the virus

was markedly attenuated, having just enough vitality to cause a slight onset of the disease, which led to a speedy recovery.

## EXPERIMENT 16

Phenol (0.5 per cent) extract of pancreas, 5 days old.

*Carabao 107.*—Known history prior to the experiment: Native Jolo carabao, 5 years and 6 months old, received at the laboratory and placed in quarantine March 3, 1917. This animal was kept under observation eighty days before it was used and did not present a high temperature or develop any symptoms of sickness during that period.

May 23, 1917, carabao 107 received subcutaneously 100 cubic centimeters of a 5-day-old 0.5 per cent phenol extract of pancreas from bull 4 (P. C. W.), which was bled to death on the first day of temperature for virulent blood, to be used in serum production.

The extract was prepared as follows:

Pancreas, 100 grams; 0.5 per cent phenol, 200 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

Carabao 107 never developed any ill effects from this injection.

June 13, which was twenty-one days after the pancreas-extract injection, carabao 107 received 50 cubic centimeters of virulent blood.

June 18, carabao 107 presented its first rise in temperature, registering, in the forenoon,  $39.8^{\circ}$  C.; in the afternoon,  $40.6^{\circ}$  C.

June 19, diarrhœa.

June 20–21, diarrhœa, not eating.

June 22, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest.

From this result it appears that the pancreas extracted in this manner is not virulent after five days.

## EXPERIMENT 17

Phenol (0.5 per cent) extract of liver, spleen, and parotid and lymph glands, 55 days old.

*Bull 4285.*—Known history prior to the experiment: Native Fuga bull, 3 years old, received at the laboratory and placed in quarantine May 8, 1917. This animal was kept under observation thirty-four days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

June 12, 1917, bull 4285 received subcutaneously 120 cubic centimeters of a 55-day-old 0.5 per cent phenol extract of liver,

spleen, and parotid and lymph glands from carabao 228 (P. C. W.). This extract was from the same lot used in experiment 12. At this time the liver extract had a slight butyric acid odor.

June 20, bull 4285 presented its first rise in temperature, registering, in the forenoon, 39° C.; in the afternoon, 40.5° C.

June 21, diarrhœa; forenoon temperature, 40° C.; afternoon, 40.6° C.

June 22-24, diarrhœa, not eating.

June 25, found dead in the morning. This animal presented typical symptoms and lesions of rinderpest.

From this result it appears possible to keep the virus in a virulent form for as long a period as fifty-five days in a 0.5 per cent phenol solution.

#### EXPERIMENT 18

Phenol (1 per cent) extract of lymph glands, 6 days old.

*Bull 4296.*—Known history prior to experiment: Native Fuga bull, 2 years old, received at the laboratory and placed in quarantine June 1, 1917. This animal was kept under observation for seventeen days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

June 18, 1917, bull 4296 received subcutaneously 100 cubic centimeters of a 6-day-old 1 per cent phenol extract of lymph glands from bulls 1036 and 1037 (P. C. W.), which were bled to death on the second day of temperature for virulent blood, to be used in the production of antirinderpest serum.

The extract was prepared as follows:

Lymph glands, 200 grams; 1 per cent phenol, 400 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was replaced in the refrigerator.

June 22, bull 4296 presented its first rise in temperature, registering, in the forenoon, 39.2° C.; in the afternoon, 41.° C.

June 24-27, diarrhœa, not eating.

June 28, died during the forenoon. This animal presented typical symptoms and lesions of rinderpest, which proves that a 1 per cent phenol solution will not destroy the virus of rinderpest in the lymph glands over a period of six days, nor does the virus appear to be attenuated by its presence.

#### EXPERIMENT 19

Phenol (1 per cent) extract of liver, spleen, cæcum, and lymph glands, 17 days old.



*Bull 4299.*—Known history prior to experiment: Native Fuga bull, 2 years old, received at the laboratory and placed in quarantine June, 1, 1917. This animal was kept under observation for twenty-five days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

The extract to be used was prepared as follows:

Liver, 100 grams; 1 per cent phenol, 200 cubic centimeters.

Spleen, 100 grams; 1 per cent phenol, 200 cubic centimeters.

Cæcum, 100 grams; 1 per cent phenol, 200 cubic centimeters.

Lymph glands, 100 grams; 1 per cent phenol, 200 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

June 25, 50 cubic centimeters of each extract were added, making a total of 200 cubic centimeters; to this mixed extract 20 cubic centimeters of a 1-1,000 per cent chlorazene solution were added, and the resulting mixture was returned to the refrigerator.

June 26, 1917, bull 4299 received subcutaneously 100 cubic centimeters of the 17-day-old 1 per cent phenol extract of liver, spleen, lymph glands, and cæcum from bull 1034 (P. C. W.), which was bled to death on the first day of temperature for virulent blood, to which the 1-1,000 per cent chlorazene solution had been added on the previous day.

July 1, 1917, bull 4299 presented its first rise in temperature, registering, in the forenoon, 39.9° C.; in the afternoon, 39.8° C.

July 2, forenoon temperature, 40.1° C.; afternoon, 40.2° C.

July 3-5, diarrhœa.

July 6, died in the forenoon, presenting good symptoms and lesions of rinderpest.

This shows that the 1 per cent phenol solution after acting seventeen days and the 1-1,000 per cent chlorazene solution after acting one day upon the mixed tissue extracts did not have any apparent detrimental effect upon the virus of rinderpest.

#### EXPERIMENT 20

Phenol (1 per cent) extract of lymph glands, 20 days old.

*Bull 4302.*—Known history prior to experiment: Native Fuga bull, 2 years old, received at the laboratory and placed in quarantine June 1, 1917. This animal was kept under observation for twenty-eight days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

June 29, 1917, bull 4302 received subcutaneously 100 cubic centimeters of a 20-day-old 1 per cent phenol extract of lymph glands from bull 1034 (P. C. W.) (see experiment 19).

July 2, this animal presented its first rise in temperature, registering, in the afternoon, 40.2° C.

July 3, forenoon temperature, 39.6° C.; afternoon, 40.2° C.

July 6-7 diarrhœa, not eating; died during the afternoon of July 7. This animal presented typical symptoms and lesions of rinderpest.

#### EXPERIMENT 21

Phenol (1 per cent) extract of liver, 21 days old.

*Bull 4303.*—Known history prior to experiment: Native Fuga bull, 3 years old, received at the laboratory and placed in quarantine June, 1, 1917. This animal was kept under observation twenty-nine days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

June 30, 1917, bull 4303 received subcutaneously 100 cubic centimeters of a 21-day-old 1 per cent phenol extract of liver from bull 1034 (P. C. W.) (see experiment 19).

This animal ran a rather erratic temperature from July 3 until death.

July 9, diarrhœa; afternoon temperature, 40° C., which was the highest temperature registered during the course of the disease.

July 10, diarrhœa.

July 11, diarrhœa, not eating; afternoon temperature so low that it could not be read.

July 12, found dead in the morning. This animal developed rather atypical symptoms, which do sometimes occur in rinderpest.<sup>(3)</sup> It presented good lesions of the disease.

#### EXPERIMENT 22

Phenol (1 per cent) extract of spleen, 21 days old.

*Bull 4304.*—Known history prior to experiment: Native Fuga bull, 2 years old, received at the laboratory and placed in quarantine June 1, 1917. This animal was kept under observation for twenty-nine days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

June 30, 1917, bull 4304 received subcutaneously 100 cubic centimeters of a 21-day-old 1 per cent phenol extract of spleen from bull 1034 (P. C. W.) (see experiment 19).

July 3, this animal presented its initial rise in temperature, registering, in the forenoon,  $39.4^{\circ}$  C.; in the afternoon,  $40.6^{\circ}$  C.

July 6–8, diarrhoea, not eating.

July 9, died, presenting good symptoms and lesions of rinderpest.

It will be noticed from the results obtained in experiments 20, 21, and 22 that the 1 per cent phenol had apparently no detrimental effect upon the virus of rinderpest when contained in the lymph glands for twenty days and in the liver and in the spleen for twenty-one days.

#### EXPERIMENT 23

Phenol (1 per cent) extract of lymph glands, 17 days old.

*Bull 4306.*—Known history prior to experiment: Native Fuga bull, 3 years old, received at the laboratory and placed in quarantine June 1, 1917. This animal was kept under observation for thirty-eight days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

July 9, 1917, bull 4306 received subcutaneously 100 cubic centimeters of a 17-day-old 1 per cent phenol extract of lymph glands from carabao 107, which died of rinderpest on the sixth day of temperature (see experiment 16).

The extract was prepared as follows:

Lymph glands, 200 grams; 1 per cent phenol, 400 cubic centimeters.

This was placed in the refrigerator for three days; it was then filtered through gauze and returned to the refrigerator.

This animal ran an atypical course of the disease. On July 10, the afternoon temperature was  $39.4^{\circ}$  C., which was the highest temperature registered.

July 13–14, diarrhoea, not eating.

July 15, found dead in the morning. This animal presented good physical symptoms and good lesions of rinderpest, but did not develop a high temperature. The disease was very acute, as the animal was dead on the morning of the sixth day after injection.

#### EXPERIMENT 24

Phenol (2 per cent) extract of spleen, 5 days old.

*Bull 4316.*—Known history prior to experiment: Native Batanes bull, 6 years old, received at the laboratory and placed in quarantine June 3, 1917. This animal was kept under observation for seventy-two days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.



August 15, 1917, bull 4316 received subcutaneously 50 cubic centimeters of a 5-day-old 2 per cent phenol extract of spleen from a bull (P. C. W.) that was bled to death for virulent blood on its second day of temperature. This spleen extract was agitated at room temperature for forty-eight hours.

The extract was prepared as follows:

Spleen, 100 grams; 2 per cent phenol, 200 cubic centimeters.

This was placed in a shaking machine and agitated continuously for forty-eight hours at room temperature. At the expiration of forty-eight hours it was placed in the refrigerator for twenty-four hours; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

August 20, afternoon temperature, 39.8° C.

August 21, forenoon temperature, 38.7° C.; afternoon, 39.8° C.

August 22, forenoon temperature, 39° C.; afternoon, 40.2° C.

August 25-26, eating little.

This animal was given 600 cubic centimeters of antirinderpest serum on August 21, 200 cubic centimeters on August 22, and 100 cubic centimeters on August 25. With the mildness of the attack and the administration of the serum the animal made a speedy recovery.

This animal was constantly exposed to animals sick with rinderpest. On September 13, 1917, it was injected with 2,000 cubic centimeters of a 7-day-old 0.75 per cent phenol extract of liver and lymph glands. It never developed the disease, proving that it had been immunized by its first attack.

September 27, 1917, it was considered hyperimmune and was bled to death for its serum.

This proves that the 2 per cent phenol and the agitation did not destroy the virus of rinderpest, but undoubtedly attenuated it to some extent.

#### EXPERIMENT 25

Phenol (2 per cent) and glycerin extract of spleen, 5 days old.

*Bull 4308.*—Known history prior to experiment: Native Fuga bull, 3 years and 3 months old, received at the laboratory and placed in quarantine June 1, 1917. This animal was kept under observation seventy-four days before it was used; it did not present a high temperature or develop any symptoms of sickness during this period.

August 15, 1917, bull 4308 received subcutaneously 50 cubic centimeters of a 5-day-old 2 per cent phenol extract of spleen, to which glycerin was added, from the bull (P. C. W.) mentioned in experiment 24.

The extract was prepared as follows:

Spleen, 100 grams.  
Glycerin, 50 cubic centimeters.  
Water, 150 cubic centimeters.  
Phenol (pure), 4 cubic centimeters.

This was placed in a shaking machine and agitated continuously for forty-eight hours at room temperature. At the expiration of forty-eight hours it was placed in the refrigerator for twenty-four hours; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

Bull 4308 did not develop any symptoms of disease from this injection.

August 29, which was fourteen days after the extract injection, this animal was injected with 50 cubic centimeters of virulent blood.

September 1, this animal presented its first rise in temperature, registering, in the forenoon,  $39.5^{\circ}$  C.; in the afternoon,  $40.1^{\circ}$  C.

It ran a rather severe course of the disease, but recovered.

This proves that the virus was destroyed in the spleen extract by the action of the 2 per cent phenol and glycerin.

Several similar experiments were tried, using liver and lymph glands, and it was found that the virus was destroyed in each case where glycerin was added.

#### EXPERIMENT 26

Phenol (2 per cent) extract of lymph glands, 8 days old.

*Bull 4335.*—Known history prior to experiment: Native Fuga bull, 3 years old, received at the laboratory and placed in quarantine August 28, 1917. This animal was used on the day of its arrival; consequently it was not under observation previous to the experiment.

August 28, 1917, bull 4335 received subcutaneously 50 cubic centimeters of an 8-day-old 2 per cent phenol extract of lymph glands from bull 1660 (P. C. W.), which was bled to death on its second day of temperature for virulent blood.

The extract was prepared as follows:

Lymph glands, 100 grams; 2 per cent phenol, 200 cubic centimeters.

This was placed in the shaking machine and agitated continuously for forty-eight hours at room temperature. At the expiration of forty-eight hours it was placed in the refrigerator for twenty-four hours; it was then filtered through gauze, and the filtrate was returned to the refrigerator.

This injection had no ill effect upon bull 4335.

September 11, 1917, which was fourteen days after the 2 per cent phenol and lymph gland extract injection, this animal received 50 cubic centimeters of a 5-day-old 0.75 per cent phenol extract of liver and lymph glands from carabao 97, which was bled to death for virulent blood on its fourth day of temperature.

September 13, bull 4335 presented its first rise in temperature, registering, in the forenoon, 39.6° C.; in the afternoon, 39.8° C.

September 14, forenoon temperature, 40.2° C.; afternoon, 40.3° C.

September 15, diarrhœa; bled to death for virulent blood, to be used in immunization work. This animal presented good symptoms and lesions of rinderpest.

This proves that the 2 per cent phenol and the agitation together destroyed the virus of rinderpest in the lymph gland extract after eight days.

Similar results were obtained by treating liver tissue in the same way and for the same length of time.

Heart and intestine extracts were found to lose their virulence in six days when treated in the above-mentioned manner.

#### DISCUSSION

From the results obtained in the foregoing experiments, it is apparent that the virus of rinderpest held in certain tissues of the body is not injured when extracted with weak solutions of phenol. From many observations that have been made in this laboratory during the past seven years, it has been noticed that the virus of rinderpest is quickly destroyed in decomposing material, either tissue or blood. On the other hand, if virulent blood is drawn under aseptic conditions and placed in sterile containers, the virus will retain its activity for five or six days. If the blood is kept in a clotted form, the virus retains its activity a few days longer. It has been shown in previous work<sup>(2)</sup> that when the large water leech (*Hirudo boyntoni* Wharton) is allowed to feed upon an animal sick with rinderpest, the virus may remain active for a period of twenty-five days inside the body of the leech. In this case the blood is kept from putrefactive organisms and also in a semianaërobic condition.

By extracting certain organs with weak phenol solutions, the activity of the putrefactive organisms is kept down to the minimum, and thus they have little or no effect upon the virus of rinderpest.

Many times a certain method will work in the hands of the originator, but when placed in other hands the same good results are not obtained. To check this, Drs. Ildefonso Patdu and



Florencio Patenia have made these extracts with no supervision from me, and the results obtained from these extracts were similar to those obtained where I had had full supervision.

To prove that these extracts would work as readily upon animals not used in the laboratory, on three different occasions extracts were given to Dr. D. W. Shaffer and Mr. Thomas L. Bean to be used as virulent material on animals that were used in the production of antirinderpest serum in the Philip C. Whitaker antirinderpest serum laboratory. Doctor Shaffer and Mr. Bean obtained as good results with the extracts as we had in the research laboratory, which proves that these extracts work as readily on animals outside as on those inside of the laboratory.

These extracts have been used in the immunization stations in the provinces, under the supervision of Dr. Stanton Youngberg, chief veterinarian.<sup>2</sup> In preparing extracts for the provinces, we use a 0.75 per cent phenol solution. For ordinary immunization work it is best not to use an extract over 15 days old, as there are other factors that enter in that are apt to delude. We have obtained a considerable number of very gratifying results with old extracts, which will be reported in a subsequent paper. On these occasions the animals presented no reaction to the injection. After a period of two weeks these animals were exposed to rinderpest by various methods, that is, by exposure to sick animals, inoculation with virulent blood, and inoculation with extracts. These animals presented no ill effects from the exposures to which they were subjected, showing that they had been immunized by the primary injection of extract.

From the result obtained by Birch on hog cholera,<sup>(1)</sup> it is possible that tissue extracts can be used as readily in that disease as in rinderpest, thereby lowering the enormous expense of obtaining virulent material in the production of antihog-cholera serum.

I wish to thank Dr. D. W. Shaffer for the privilege of securing various tissues used in these experiments from animals used by him in obtaining virulent blood in the process of making anti-rinderpest serum.

#### CONCLUSIONS

1. From the results obtained in experiments 1 and 2, it is evident that water extracts of the liver, spleen, and lymph glands, 3 days old, are highly infectious to susceptible animals.

2. From the results obtained in experiments 3 and 4, it is

<sup>2</sup> *Phil. Agr. Rev.* (1917), 10.

evident that a 0.5 per cent phenol extract of liver, spleen, and lymph glands, 5 days old, is highly infectious to susceptible animals.

3. From the result obtained in experiment 5, it is evident that a 0.5 per cent phenol extract of heart muscle, 5 days old, is highly infectious to susceptible animals.

4. From the result obtained in experiment 6, it appears that the skeletal muscle is not a suitable tissue for making extracts in the case of rinderpest.

5. From the results obtained in experiments 7, 10, 11, 12, 13, 14, 15, and 17, it is proved that a 0.5 per cent phenol extract of liver, spleen, and lymph glands can hold the virus of rinderpest in a virulent form for periods of time varying from eight to fifty-five days.

6. From the result obtained in experiment 8, it is evident that a 0.5 per cent phenol extract of cæcum and of colon, 5 days old, is highly infectious to susceptible animals.

7. From the result obtained in experiment 9, it is apparent that the larynx, pharynx, and base of tongue are not suitable tissues for making extracts in the case of rinderpest.

8. From the result obtained in experiment 16, it is apparent that the pancreas is not a suitable tissue for making extracts in the case of rinderpest.

9. From the results obtained in experiments 18, 20, and 23, it is evident that a 1 per cent phenol extract of lymph glands, 6, 20, and 17 days old, respectively, is highly infectious to susceptible animals.

10. From the result obtained in experiment 19, it is evident that a 1 per cent phenol extract of liver, spleen, cæcum, and lymph glands, 17 days old, is highly infectious to susceptible animals.

11. From the result obtained in experiment 21, it is evident that a 1 per cent phenol extract of liver, 21 days old, is virulent to susceptible animals.

12. From the result obtained in experiment 22, it is evident that a 1 per cent phenol extract of spleen, 21 days old, is virulent to susceptible animals.

13. From the result obtained in experiment 24, it is evident that a 2 per cent phenol extract of spleen, 5 days old, is infectious to susceptible animals.

14. From the result obtained in experiment 25, it appears that when glycerin is added to a 2 per cent phenol extract that has been agitated for forty-eight hours the virus of rinderpest is readily destroyed.

15. From the result obtained in experiment 26, it appears that in a 2 per cent phenol extract of lymph glands, 8 days old, the virus of rinderpest is destroyed.

16. From certain results mentioned in the discussion, it is advisable to use a 0.75 per cent phenol extract not over 15 days old.

17. From the results obtained in working with rinderpest, it is very plausible that similar or even better results may be obtained with the virus of hog cholera along these lines.

18. The tissues best adapted for this work are the liver, spleen, lymph glands, heart, fourth stomach, cæcum, and colon.

#### REFERENCES

1. BIRCH, R. R. Hog cholera transmission through infected pork. *Journ. Am. Vet. Med. Assoc.* (1917), n. s. 4.
2. BOYNTON, W. H. Duration of the infectiveness of virulent rinderpest blood in the water leech, *Hirudo boyntoni* Wharton. *Phil. Journ. Sci., Sec. B* (1913), 8, 509.
3. IDEM. An atypical case of rinderpest in a carabao. *Ibid., Sec. B* (1914), 9, 45.
4. IDEM. Experiments on the treatment of rinderpest with various drugs. *Phil. Agr. Rev.* (1917), 10, 272.



NOTE ON THE USE OF ORGAN EXTRACTS IN PLACE OF VIRU-  
LENT BLOOD IN IMMUNIZATION AND HYPERIM-  
MUNIZATION AGAINST RINDERPEST <sup>1</sup>

By WILLIAM HUTCHINS BOYNTON  
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In a paper appearing in this number <sup>2</sup> it will be noted that after an animal has been bled to death for virulent rinderpest blood weak phenol extracts can be made from the liver, spleen, lymphatics, heart, and intestinal tract and that these extracts are as potent as virulent blood.

One of the problems in the production of antirinderpest serum is the reduction in the cost of producing virulent material to be used in hyperimmunization. Various methods have been used with more or less success. Nicolle and Adil-Bey<sup>(3)</sup> were the first to develop a method by which the virulent material could be increased. When an infected animal presented symptoms of diarrhoea, they introduced into the peritoneal cavity a mixture composed of 3 volumes of normal saline solution and 1 volume of a slightly alkaline solution of Martin's peptone. They introduced 6 liters of this material into yearling cattle (the quantity varying according to the size of the animal), and after three hours the animal was bled to death, the peritoneal cavity was opened, and the fluid was aspirated. After allowing this to coagulate, the clear liquid was drained off and used. The fluid thus obtained gave an increase in virulent material, which was used with success in hyperimmunization.

Ruediger<sup>(4)</sup> obtained equal results using normal saline solution, which he allowed to remain in the peritoneal cavity from one to two hours before bleeding the animal to death and withdrawing it. The same author<sup>(5)</sup> also used a 5 per cent sodium citrate solution with equal results.

Holmes<sup>(1)</sup> diluted the virulent blood with an equal volume of potassium citrate solution and claims the diluted blood gave better results than undiluted defibrinated blood.

Martoglio<sup>(2)</sup> has developed the latest method, in which he

<sup>1</sup> Published in *Phil. Agr. Rev.* (1917), 10, 448.

<sup>2</sup> *This Journal, Sec. B* (1918), 13, 127; also *Phil. Agr. Rev.* (1917), 10, 410.

claims to increase the virulent material about 70 per cent. His technic is as follows:

When the infected bovine presents the buccal lesions, usually at the end of the fourth or fifth day of the fever, less commonly at the end of the third, sixth, or seventh, it is immobilized in the stocks and intubed in the jugular and the carotid on the same side. The jugular is put in communication with a capacious glass receptacle placed on a level with the head of the animal and containing saline solution, sterilized and at a temperature of 38° to 39°C., leaving the outlet tube of rubber closed by compression of pincers. \* \* \* The carotid is put in communication with the receptacle which receives the pest blood, and the bleeding begins.

When the convulsions preceding the death-struggle begin, the bleeding should stop. The assistant shuts the tube for drawing the blood with a clamp and opens the tube admitting the sodium-chloride solution; immediately the serious symptom-complex changes, the muscular contractions begin to cease, the respiration and pulse that were accelerated become regular and the animal when it has received about as much solution as it has lost blood, enters a period of calm.

We usually inject enough solution to make two and a half times the volume of blood taken, and without ill results. \* \* \* The operation over the animal returns to its shed without assistance. After a lapse of about 5 or 6 hours the animal is bled from the same carotid, this time until it dies.

By this method Martoglio claims to wash out the blood vessels and lymphatic system and obtain a potent virus.

Dr. Stanton Youngberg, chief veterinarian, Bureau of Agriculture, and Dr. D. W. Shaffer, formerly in charge of the Philip C. Whitaker antirinderpest serum laboratory, Manila, have been using a simple method of slightly increasing the production of virulent blood as follows: The injected animal is bled from 2 to 4 liters of blood, depending upon its size, on the second day of temperature; the animal is then allowed to stand overnight, during which time the body has an opportunity to replace the volume of the blood lost; on the following day it is bled to death. In the final bleeding practically as much blood in bulk is obtained as would be procured in a single bleeding, which gives an increase in virulent material corresponding to the amount obtained at the initial bleeding.

#### TISSUE-EXTRACT METHOD

Any of the above-mentioned methods can be utilized and an enormous increase of virulent material still be obtained by extracting the organs in a weak phenol solution. To illustrate this point, we shall consider the data obtained from an animal of ordinary size, which was bled to death and from whose tissues extracts were made.

Batanes bull 4318, bled to death August 24, 1917.

Amount of virulent blood obtained, 9,000 cubic centimeters.

Weight of organs from which extracts were made:

	Grams.
Liver	1,735
Spleen	350
Lymphatics	260
Fourth stomach	320
Cæcum and colon	2,220
Heart	680
Total	5,655

These organs were passed through a meat grinder and placed in twice their bulk of a 0.75 per cent phenol solution; that is, the cæcum, colon, and fourth stomach were first thoroughly washed free from faecal matter, then placed in 5 per cent phenol solution for from five to ten minutes, after which they were placed in a large container of boiled water, which was cooled to at least 37° C. These tissues were then allowed to soak in this water for a few minutes to dilute the phenol that remained intact (by this method a greater percentage of the bacteria on the surface of the intestinal mucosa is destroyed). Following this treatment the tissue was weighed, passed through the meat grinder, and treated in a manner similar to that adopted for the other tissues (5,655 grams of tissue from this animal  $\times 2 = 11,130$  cubic centimeters, the amount of phenol solution that should be added). This material was allowed to extract for three days in the refrigerator, being thoroughly agitated three or four times each day. At the expiration of this period it was filtered through gauze to separate the coarse material, and the filtrate was returned to the refrigerator, ready for use.

From the above-mentioned animal about 11 liters of extract filtrate were obtained, plus the 9 liters of blood, which makes a total of 20 liters of virulent material; under ordinary conditions but 9 liters would have been secured.

If this animal had been handled by the method advanced by Martoglio, a still greater amount of virulent material would have been obtained. Considering that Martoglio obtains a 70 per cent increase in the virulent blood, it would bring the total up to 26,300 cubic centimeters, which would practically triple the output of virulent material from this animal, providing it had been merely bled to death.

Both simultaneous immunization and hyperimmunization have been accomplished with these tissue extracts at the laboratory and in the immunization stations in the provinces.



Doctor Youngberg had the extracts used in simultaneous immunization of carabao at the immunization station at Lubao, Pampanga Province, Doctor Topacio doing the work. These extracts were tried on two different sets of animals.

July 22, 1917, seventeen head of carabao were brought to the station for immunization and were injected with mixed liver, spleen, and lymph gland extract in a 0.5 per cent phenol solution, 5 days old. The doses administered and the final results were as follows: Eight animals received 5 cubic centimeters each of this extract, five animals giving good reactions; four animals received 10 cubic centimeters each of the extract, all of them giving good reactions; two animals received 15 cubic centimeters each of the extract, neither reacting; two animals received 20 cubic centimeters each of the extract, one reacting; one animal received 25 cubic centimeters of the extract without reacting. On August 5, 1917, all the animals that did not react were injected with 25 cubic centimeters of virulent blood, and none of them developed the disease, proving them to be immune. When these animals were injected with the extract, they also received from 250 to 600 cubic centimeters of antirinderpest serum, the amount of serum administered depending upon the size of the animal.

August 17, 1917, fifteen head of carabao, brought into the station for immunization, received 5 cubic centimeters each of an 8-day-old liver extract that had been prepared as follows: Two hundred grams of liver from an animal bled to death on the second day of temperature were passed through a meat grinder, and 400 cubic centimeters of a 0.75 per cent phenol solution were added to it. This material was placed in the refrigerator and thoroughly agitated three or four times a day. After three days' extraction it was filtered through gauze, and the filtrate was returned to the refrigerator, where it was kept until it was 7 days old. The extract was then shipped to the Lubao immunization station for use in the above injections. Of these fifteen animals, seven developed good reactions. Each of these animals received from 400 to 600 cubic centimeters of antirinderpest serum, depending on its size, at the same time the extract was injected. One of the reacting animals that received 400 cubic centimeters of antirinderpest serum succumbed to the disease, while the others made good recoveries. The animals that did not react were injected with 25 cubic centimeters of virulent blood on September 2, 1917, and one developed the disease from the second injection. There is a possibility that this animal did not contract the disease from the extract injec-

tion on account of a slight fault in the technic of administering it. Since the skin of a carabao is thick, it is difficult to use a small injection such as 5 cubic centimeters and be sure one has good penetration of the virus. When working with this type of animal, it is best to give at least 10 cubic centimeters at an injection. If but 5 cubic centimeters of the material is desired, it can be easily diluted to 10 cubic centimeters with 0.85 per cent sodium chloride solution without affecting the activity of the virus, and in this way the necessary amount of material is available.

The animals on which the extract was used were the ordinary type one has to handle in the immunization stations, as they were obtained from localities where rinderpest had been present for a number of years, and many of those brought to the station had passed through the disease by natural contact. Since at present there is no way of identifying the immunity, all animals are subjected to the same treatment. This accounts for the high percentage of nonreactors obtained in this work. From the results obtained by the use of extracts, Doctor Youngberg states that it has the same efficiency as the most potent virulent blood. With a strong strain of virulent blood he usually obtains about 50 per cent reactors on the first injection. With the extract, slightly over 50 per cent of reactions were obtained, or in other words it picked out all the susceptible animals but one. The possible reason for this one not becoming infected from the extract has been mentioned.

In using the extract for hyperimmunization, we have obtained some very satisfactory results, but there have been a few instances where the massive injection of this highly virulent material has resulted in the death of the animal. The possible causes for this will be discussed in connection with these animals.

The first hyperimmunization work with tissue extracts was accomplished by Doctor Patdu, upon Chinese cattle. These animals were imported to the Philippines to be used as work animals. Before they could be shipped to the provinces, they had to be immunized against rinderpest, which was accomplished at the quarantine station.

Fourteen of these Chinese cattle that had passed through an attack of rinderpest during the immunization process were hyperimmunized with fixed tissue extract obtained from three different animals. These extracts were prepared in 0.5 per cent phenol and were 5 days old. Thirteen of the animals received 1,500 cubic centimeters each of this extract, and one received 1,200 cubic centimeters. None of these animals developed any serious

effects from these injections. They had a slight temperature the day following the injections, which soon subsided to normal. After several days these animals were bled approximately 4 liters each. The serum thus obtained has been used in the immunization stations with good results.

In view of the results obtained in hyperimmunizing the Chinese cattle with the extracts, Doctor Youngberg had the extract tried on some animals in the provincial immunization stations. The extract was prepared at the research laboratory, using liver, spleen, lymphatics, and heart in a 0.75 per cent phenol solution. On the fourth day of extraction this material was filtered through gauze and placed in 15-liter demijohns. When it was 6 days old, it was taken by automobile to the San Fernando and the Apalit immunization stations. On the following day Dr. C. H. Leavitt, in charge of the San Fernando immunization station, injected five animals with 2,000 cubic centimeters each of this extract, and Dr. C. H. Decker, in charge of the Apalit immunization station, injected four animals with 2,000 cubic centimeters each.

Doctor Leavitt states that a short time after the injection the animals became stiff, stopped eating, and in the course of a few days they presented the appearance of being paralyzed. All five of the animals injected died. Doctor Decker had a somewhat similar experience and lost two of the four animals.

We then tried the same type of extract at the laboratory on two animals that had recently recovered from rinderpest. These animals were each injected with 2,000 cubic centimeters of a 7-day-old extract in 0.75 per cent phenol solution. In contrast to the above extracts this material had been kept in the refrigerator up to the time of injection. These animals developed a pronounced œdema in the pendent portion of the body, which completely subsided within three days by the aid of slight massaging and the application of warm water each morning. Within four days these animals were in normal condition and never presented any ill effects from the injections up to the time they were bled to death for serum.

From the results thus obtained it is evident that small Fuga and Batanes cattle can withstand 2,000 cubic centimeters of this extract at one injection without any serious disturbance.

A further test was made in trying to locate the cause of the losses incurred by Doctors Leavitt and Decker. In this experiment the extracts were made in a manner similar to that followed for those described above. On the sixth day they were placed in a demijohn and taken to San Fernando, Pampanga, by automobile and returned to the laboratory the following morning.



This was done to expose the extracts to climatic conditions in the same way that the extracts used by Doctors Leavitt and Decker had been exposed.

Two thousand cubic centimeters of this material were injected into a Batanes bull that had recently recovered from rinderpest. This animal developed an œdema similar to that developed by the others. By massaging and giving a warm bath the œdema had practically subsided by the fourth day. On the morning of the fifth day after injection this animal could not rise to its feet, but continued to ruminate and ate a little during the forenoon. In the evening it was practically paralyzed and was found dead the next morning. From the results obtained in this experiment and those obtained by Doctors Leavitt and Decker it is evident that by exposing these extracts to the climatic conditions existing in the Philippines for a period of twenty-four hours they pass through certain chemical changes, which are very detrimental to animals receiving the extracts in large quantities. The exact changes have not been determined, but they appear to be protein decomposition or botulism toxin.

We are doing further work on trying to eliminate the small particles of tissue that pass through the gauze, by first filtering the extract through gauze, then through a layer of cotton (method similar to that used in filtering agar), and finally passing it through filter paper. By this method we obtain a slightly turbid, dark amber-colored liquid. A small Fuga bull that recently recovered from rinderpest has been injected with 2,000 cubic centimeters of this material, which was kept in the refrigerator up to the time of injection. This material caused a much milder œdema, which practically subsided in two days, and the animal suffered no apparent ill effects. There was a slight elevation of temperature for two days, but the animal continued to eat well and looked bright.

#### CONCLUSIONS

1. Considering the results thus far obtained, it is evident that tissue extracts from animals suffering with rinderpest are just as potent as virulent blood when used in simultaneous immunization work.

2. Any of the methods advocated for increasing the production of virulent material can be utilized, after which the organs can be extracted, thereby obtaining a much greater increase in quantity.

3. By using Martoglio's method and extracting the organs, the output of virulent material is practically tripled.

4. If the extracts are kept at a temperature of approximately 15° C., they can be used with safety in 2,000 cubic centimeter doses for hyperimmunization.

5. Considering our results up to date, the extracts should not be given in massive injections if they have been exposed for a period of eighteen hours to the climatic conditions found in the tropics.

6. These extracts can be produced so easily that this method can be used in any immunization station.

7. Considering the similarity of hog cholera to rinderpest, this method should be as applicable in that disease as it is in rinderpest, thereby reducing the enormous cost of the virus.

#### REFERENCES

1. HOLMES, J. D. E. Experiments carried out to test the susceptibility of cattle from several districts in India, and on improved methods of rinderpest serum production. *Indian Civ. Vet. Dept.* No. 3, 98-205.
2. MARTOGLIO, F. On the technique for the production of serum against rinderpest. Utilization of the liquid of (blood) vessel lavage as antigen. *Memoir of the Eritrea Sero-vaccine Institute.* Asmara (1915), No. 1.
3. NICOLLE and ADIL-BEY. Etudes sur la peste bovine. *Ann. d. l'Inst. Pasteur* (1901), 715.
4. RUEDIGER, E. H. Filtration experiments with virus of cattle plague. *Phil. Journ. Sci., Sec. B* (1908), 3, 165.
5. IDEM. A reduction in the cost of anticattle-plague serum. *Ibid., Sec. B* (1908), 3, 407.

## COMPARATIVE STUDY ON NATURAL HEMOLYSINS IN INACTIVATED HUMAN AND MONKEYS' SERUM <sup>1</sup>

By CARLOS MONSERRAT

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The absence in human serum of natural hemolytic amboceptor toward the red corpuscles of the monkey has been mentioned in a previous communication.<sup>2</sup>

This study suggested the present experiments, in which twenty-three additional samples of human sera were tested with regard to their content of antimonkey natural hemolytic amboceptor. All these samples, showing a lack of antimonkey hemolytic amboceptor, behaved in the same manner as the forty samples of human sera previously tested.<sup>3</sup>

Kolmer and Casselmann <sup>4</sup> have recently studied the hemolysins in inactivated human serum and found that the said serum contains natural hemolysins toward red cells of the following animals: Sheep, dog, calf, goat, pig, rat, chicken, horse, rabbit, and guinea pig.

On account of our suggestion to substitute, with advantage, monkeys' corpuscles for human red cells in performing the complement fixation test for diagnosis of syphilis, it seemed of interest to compare human serum with that of the monkey with regard to hemolytic amboceptor toward red cells of some of the animals used by the above-mentioned authors.

In Table I there are recorded the results obtained by testing twenty-three inactivated human sera and one monkey's serum for hemolysins against human red corpuscles and those of the monkey, the sheep, the horse, the cow, the goat, the carabao, and the guinea pig.

*Technic.*—The sera were heated between 55° and 56° C. for thirty minutes. The amount used in the test was 0.2 cubic

<sup>1</sup> Received for publication November, 1917.

<sup>2</sup> *This Journal, Sec. B* (1917), 12, 249.

<sup>3</sup> Loc. cit.

<sup>4</sup> *Journ. Inf. Dis.* (1915), 16, 441.



centimeter. To each tube were added 1.5 cubic centimeters of physiologic salt solution (0.9 per cent) and 0.5 cubic centimeter of a 4 per cent red-cell suspension. The tubes were allowed to stand at room temperature for one hour, and during this time each tube was frequently shaken. After one hour of exposure to room temperature, 0.5 cubic centimeter of ten times diluted complement was added, using the pooled sera of three guinea pigs. The tubes were placed in the incubator for another hour, and then the results were recorded.

It is evident from the results of these tests (Table I) that none of the human sera showed the presence of natural hemolysins against human, monkeys', horses', and guinea pigs' red corpuscles, but the majority of the same sera contained hemolysins for sheep's and goats' corpuscles and in a slight degree for cows', carabaos', and rabbits' red cells.

The one monkey's serum showed lack of hemolysins against human, monkeys', horses', cows', carabaos', rabbits', and guinea pigs' corpuscles, and like the human sera this one serum contained a great amount of natural hemolysins against sheep's and goats' corpuscles.

The human, as well as the one monkey's serum, showed a strong agglutination of carabaos' and rabbits' red cells. As our first experiment was carried out with only one sample of one monkey's serum, we tested in a second experiment the sera of five different monkeys. These sera gave identical results with the one sample previously tested, as is shown in Table II.

Having established the fact that human and monkeys' sera behave in a similar way with regard to hemolysins toward red cells of various animals, we proceeded in the next experiment to test the sera of these animals, including that of man and of monkey, with regard to the presence or absence of natural hemolytic amboceptor toward the red cells of each of the animals, including man. The results of these tests are evident from Table III. The technic applied in this experiment was the same as in previous tests, except the amount of serum used, which was decreased to 0.1 cubic centimeter.

It is evident from the results of the tests given in Table III that monkeys' serum behaves in the same way as human serum does with regard to natural amboceptor toward the various red corpuscles used in the test. Furthermore human red cells and those of a monkey behave in practically the same way when exposed to the action of inactivated sera of various animals and guinea pigs' complement.

One striking thing, which may be of interest, is the finding that rabbits' serum contains no hemolytic amboceptor for human corpuscles, while it has a large amount of natural hemolytic amboceptor for monkeys' red corpuscles. Monkeys' red cells behave in that respect in a similar way as those of the sheep, the horse, and the goat. This finding probably explains the fact that artificial antimonkey amboceptor of as high titer as that of the sheep, the goat, and the horse can be produced.

This question is being studied in further experiments now under way.

The object of these experiments is to test more than one rabbit's serum and to study the influence of immunization on the amount of natural hemolytic amboceptor.

#### CONCLUSIONS

1. Inactivated human sera contain no natural amboceptor for monkeys' red corpuscles, but a great percentage of human sera contains a large amount of hemolytic amboceptor for sheep's and goats' corpuscles.

2. Inactivated monkeys' serum contains no natural amboceptor for human red corpuscles, but contains a large amount of natural hemolytic amboceptor for sheep's and goats' corpuscles.

3. Natural hemolytic amboceptor of human and monkeys' sera are almost identical, not having the same relation in this respect with the sera of the sheep, the horse, the cow, the goat, the rabbit, and guinea pig.

4. The serum of the rabbit (one animal) shows hemolysins for the corpuscles of the sheep, the horse, the monkey, and the goat.

TABLE I.—Showing comparative tests of human sera and one monkey's serum.

[Inactivated serum, 0.2 cubic centimeter; guinea pigs' complement, 0.05 cubic centimeter; —, no hemolysis; +, trace of hemolysis; +2, slight hemolysis; +3, moderate hemolysis; +4, strong hemolysis; +5, almost complete hemolysis; +6, complete hemolysis; a, agglutination.]

0.5 cc. of 4 per cent suspension of sensi- tized red corpuscles of—	Mon- key. I	Human—										
		1	2	3	4	5	6	7	8	9	10	11
		M. A.	B. R.	G. N.	G.	B. L.	A. K.	D.	A. A.	M. C.	T. O.	F. B.
Man .....	—	—	—	—	—	—	—	—	—	—	—	—
Monkey .....	—	—	a	a	—	a	—	—	a	a	—	—
Sheep .....	+6	+5	+3	a	—	—	+5	—	+6	+6	+3	+3
Horse .....	—	—	a	a	—	—	—	—	a	—	—	—
Cow .....	—	—	—	—	—	—	—	—	+	—	+	+3
Carabao .....	a	a	a	a	a	a	a	a	a	a	a	a
Goat .....	+5	+6	+3	—	—	—	+3	—	+5	+6	+6	+6
Rabbit .....	a	a	a	a	a	a	a	a	a	a	a	a
Guinea pig .....	—	—	—	a	—	—	—	—	a	—	a	—

0.5 cc. of 4 per cent suspension of sensi- tized red corpuscles of—	Human—												
	12	13	14	15	16	17	18	19	20	21	22	23	
	I. S.	F. F.	V. G.	S. S.	L. B.	F. C.	A. R.	F. F. C.	Y. B.	E. B.	U. S.	C.	
Man .....	—	—	—	—	—	—	—	—	—	—	—	—	
Monkey .....	—	—	—	—	—	—	—	—	—	—	—	—	
Sheep .....	+3	+6	+6	+6	+3	+3	+6	+6	+6	+5	+6	—	
Horse .....	—	a	—	—	—	—	—	—	—	—	—	—	
Cow .....	—	—	—	—	—	—	—	—	—	+3	—	—	
Carabao .....	a	a	a	+2a	a	a	+2a	a	a	a	a	a	
Goat .....	+2	+6	+6	+6	+3	+3	+6	+6	+5	+3	+6	—	
Rabbit .....	a	a	a	+2a	a	a	a	a	a	+2a	a	a	
Guinea pig .....	a	—	—	a	—	—	—	—	—	a	—	—	

TABLE II.—Showing tests of five different samples of monkeys' serum.\*

[Inactivated monkeys' serum, 0.2 cubic centimeter; guinea pigs' complement, 0.05 cubic centimeter.]

0.5 cc. of 4 per cent suspension of sensitized red corpuscles of—	No. 1.	No. 2.	No. 3.	No. 4.	No. 5.
Man .....	—	—	—	—	—
Monkey .....	—	—	—	—	—
Sheep .....	+5	+4	+4	+5	+5
Horse .....	—	—	—	—	—
Cow .....	—	—	—	—	—
Goat .....	+4	+2	+2	+5	+5
Rabbit .....	a	a	a	a	a
Guinea pig .....	—	—	—	—	—

\* See Table I for abbreviations and signs.



TABLE III.—*Showing cross tests of sera and red cells of various animals, including man.<sup>a</sup>*

[Inactivated serum, 0.1 cubic centimeter; guinea pigs' complement, 0.05 cubic centimeter.]

0.5 cc. of 4 per cent suspension of sensitized red corpuscles of—	Hu- man.	Mon- key.	Sheep.	Horse.	Cow.	Goat.	Rabbit.	Guinea pig.
Man .....	—	—	—	—	—	— <sup>a</sup>	—	—
Monkey .....	—	—	—	—	—	—	+4	—
Sheep .....	—	+6	—	—	+3	—	+6	—
Horse .....	—	—	+	—	+2 <sup>a</sup>	—	+5	—
Cow .....	—	+	—	—	—	—	—	+
Goat .....	—	+5	—	—	+5	—	+4	—
Rabbit .....	— <sup>a</sup>	+2 <sup>a</sup>	—	—	—	— <sup>a</sup>	—	—
Guinea pig .....	—	—	—	—	—	—	—	—

<sup>a</sup> See Table I for abbreviations and signs.



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STUDIES ON CRYPTOPLASMIC INFECTION. I, DEVELOPMENT  
OF A CRYPTOCOCCUS IN CULTURES FROM AN UNCLAS-  
SIFIED CHRONIC PHILIPPINE ULCER<sup>1</sup>

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FIVE PLATES

GENERAL HYPOTHESIS

In the pathology of fungous infections of the deeper tissues there are sometimes noted features difficult to explain on the basis of the recognized factors of infection. Having been interested by certain such observations on the American "blastomycosis," so-called, I was led to undertake a study of mycotic lesions as they occur in the Philippine Islands. From the outset there were encountered difficulties and peculiarities, and a number of observations have been made that, for their explanation, have led to the formulation of a tentative hypothesis that departs radically from orthodox views. Though it is impossible as yet fully to define these observations, it seems desirable to outline their chief features in order to indicate the line of investigation to which they have led.

Essentially it appears as if the differentiated, demonstrable parasitic element in a mycotic lesion may give rise to a derivative substance, morphologically not differentiated and perhaps even quite amorphous, that, unlike the known "toxins," soluble

<sup>1</sup> This article, though preceded by three others having to do with certain infectious fungi and the lesions caused by them [*Journ. Inf. Dis.* (1916), 18, 618; *Arch. Int. Med.* (1916), 18, 103; and *This Journal, Sec. B* (1916), 11, 267], is the first paper of a contemplated series based on a group of more or less related observations and experimental studies, by the sum total of which it is hoped that the general hypothesis here outlined may be established.



or insoluble, is by itself more or less viable and may be capable, to a certain extent at least and under certain conditions, of persisting as such and even of increasing or growing.

The hypothetical substance or body (to designate which the term "cryptoplasm" seems appropriate), though it may play a more or less important part in the pathogenic expression of the infection, is probably in some cases dependent upon the presence of the original differentiated parasitic element for its continuous replacement and thus for its perpetuation in the lesion, in which case it would be successfully resisted by the host in the absence of the formed element. In other cases, however, it may be able to perpetuate itself and maintain the lesion. Reversion to the differentiated parasitic form may be possible in some cases, under proper conditions; in other cases, and under ordinary conditions, it probably does not occur.

There is evidence that the cryptoplasm is, or may become, intraleucocytic and that, though morphologically indefinable from the protoplasm of the host cell, it may there exert special influences, producing special results, and may sometimes even be indirectly demonstrable therein by cultural methods as a formed, though not necessarily as a specifically differentiated element.

Such a substance, it is evident, might sometimes be of greater immediate importance from the viewpoint of pathogenicity than its formed generator. Should it replace the latter entirely in a progressive lesion, efforts to demonstrate or isolate the causative agent of that lesion by usual methods would of necessity fail. Under such conditions the postulates of Koch would be impossible of fulfillment, since these are based on the essential simplicity of pathogenic organisms and of their *modus operandi*, on the integrity of form of the invading organism, on its persistence during parasitism and final recoverability in cultures as such, and on the suitability or adaptability of the organism as such to both parasitic and saprophytic life.

The above hypothesis is based on the results of but two years' work and, no doubt, will be more or less modified by future studies. As stated, it has been constructed to cover a group of more or less clear-cut observations. Though much of it cannot as yet be proved, it is what appeals to me as the more conservative of two possible interpretations, the other of which will not be discussed at this point.

#### INTRODUCTION

In the present report it is desired to describe particularly certain features observed in the course of a cultural study of

material from a series of chronic, apparently mycotic ulcers, in the fresh material from which no parasitic element could be detected. These features have to do with the appearance in the cultures of a differentiated fungous organism, apparently as a result of certain peculiar changes in the very substance of the cells of the infected material.

This is a phenomenon so peculiar, so unlike anything known to have been observed in the study of such infections, and so radically different from the behavior of infectious organisms as generally understood, that it quite overshadows in its possible broader significance whatever interest attaches to the pathologic condition from which the material was obtained. It constitutes an essential contribution to the general hypothesis outlined above. At the present time it is impossible to do more than describe the findings in detail and discuss the apparent explanation. Direct confirmation cannot as yet be had; that must depend upon future studies. Indirect evidence supportive of the explanation suggested has been found in other studies yet to be reported.

Chronic ulcerations of types not definitely classifiable are not uncommon in the Philippine Islands and, apparently, in other parts of the tropics. Many of these are doubtless assignable to syphilis. Chronic ulcers, often associated with destructive lesions, also occur as a late manifestation of yaws in districts where this disease is endemic. There still remain, however, ulcers that are not assignable with probability to any recognized cause; a small group of such lesions is the basis of this report.

Few reports of investigations of such lesions in the Philippine Islands are to be found. Strong<sup>2</sup> described three types of ulcers, in sections from one of which he found bodies that he concluded were fungous. The etiology of the other two remained obscure.

Stitt,<sup>3</sup> before the Sixth International Dermatological Congress (1907), said that failing to find tropical ulcer as described in the textbooks he had concluded that it did not occur in the Philippines, though ulcers due to neglected wounds and to the infective granulomata were common. In Guam, also, none were found, though extensive and mutilating ulcerations were frequently seen. Among Americans, for the most part sailors in the Naval Hospital at Cañacao, Philippine Islands, he observed two distinct types. One began as a painless swelling that later began to ooze, resolving into a chronic ulcer, sometimes under-

<sup>2</sup> Strong, R. P., *This Journal* (1906), 1, 91.

<sup>3</sup> Stitt, E. R., *Journ. Cut. Dis.* (1908), 26, 103; also ref. *Journ. Am. Med. Assoc.* (1907), 49, 1305.

mined, sometimes punched out. Deep scrapings showed no pyogenic organisms and practically no polymorphonuclear leucocytes. Lymphocytes predominated with many large cells, 20 to 30 microns in diameter, some of which looked as if they were full of small circular bodies, which, however, showed no chromatin staining after Giemsa's stain. Healing was very slow, and treatment was unsatisfactory. The second type was characterized by an acute exudate with a dirty membrane and irregular-staining diphtheroid bacilli.

Shattuck,<sup>4</sup> at the same meeting, reported a series of 35 cases of chronic Philippine ulcers, none of which was a typical "tropical ulcer" (phagedenic); all were of obscure causation. On clinical grounds solely, microscopic findings being negative or ambiguous, he concluded that there were four types. Syphilis was probably a factor in a few cases and possibly so in the great majority; others were not diagnosed. The diagnosis of probable syphilis was arrived at by exclusion, not by positive findings, and in spite of atypical features. In the group of 19 cases from Catbalogan, Samar, the resemblance between the different individual cases was very striking and pointed to a common etiology, although the lesions ranged from simple ulcers to contractures and loss of toes and bone destruction. The possibility that any of these may have represented late yaws was not discussed.

#### GENERAL DESCRIPTION

The present report includes six cases that were seen in the clinic of the Sanitary Survey Commission No. 2 of the Philippine Health Service, during its survey of Hagonoy, Rizal, by Dr. T. P. Corpus, the Commission surgeon, acting under instructions to note particularly all cases suggestive of blastomycosis or any allied condition. Some were seen by me once, others twice. Material for study was collected each time, and on the second occasion photographs of four of them were obtained. More extensive study of the cases themselves was prevented by prevailing circumstances.

#### GROSS LESIONS

The lesions varied greatly in duration, extent, and activity, yet presented features that suggest a common etiology. However, they possessed no peculiarity that could differentiate them distinctly as a clinical entity. They developed subcutaneously, sometimes as a pustule, usually as a firm nodule that broke

<sup>4</sup> Shattuck, R. R., *This Journal*, Sec. B (1907), 2, 551.



down after an indefinite period. Usually they were not in themselves painful, though sensitive to manipulation.

Shallowness of the ulcer itself and fairly extensive undermining of the skin along an advancing border (as in fig. 2, etc.) were features of the superficial lesion. Frequently, however, deeper structures had been more or less promptly invaded, with disturbance of function and, in one case (fig. 5), shortening of an affected member. Fusion of separate ulcers or progression of lesions irregularly outward with cicatrization of the older central area had given rise to considerable irregularity of outline of the affected areas. Induration of the advancing margin was usually noticeable, though skin that had become well undermined might on the surface still appear practically normal. The floor of the ulcer was usually covered with a thin layer of pale necrotic material. Beneath this was a shallow, pale granular layer of invaded subcutaneous tissue, more or less soft and friable, sometimes exuberant and vascular, bleeding freely on manipulation. Those lesions invading deeper structures could not be investigated.

The process had sometimes subsided greatly only to break out afresh, with activation of indolent lesions and development and comparatively rapid extension of new foci. In other cases it had progressed slowly for months and years. It had usually resulted in more or less destruction and cicatrization of the soft parts (fig. 6), though in one case there was considerable thickening of the foot, somewhat suggestive of a mycetoma.

I am informed that administration of potassium iodide in large doses caused improvement in the cases so treated; however, the period of observation after treatment was begun was too short for results to be apparent. Conditions have prevented subsequent observation.

#### MICROSCOPIC FINDINGS

*Smears.*—Nothing has been found in stained smears to which the causative rôle could be possibly assigned. In those from superficial levels bacteria of different types are found, though they are not numerous. Polymorphonuclear leucocytes usually predominate. In smears from the deeper levels bacteria are scarce when found, and cells of the lymphoid type usually predominate. Eosinophiles are sometimes numerous.

The only objects of interest observed with any regularity are small, round or oval bodies, staining deeply with Loeffler's blue or Giemsa's stain, apparently produced by fragmentation of leucocytes. In some preparations they are scarce, and in others

they are fairly numerous. Most of them measure 2 to 3 microns in diameter. They usually lie singly; sometimes they lie in pairs. Occasionally a medium-sized body is surrounded by a zone of faintly staining protoplasm.

The formation of bodies presumably identical may be sometimes seen in a polymorphonuclear leucocyte the nucleus of which is breaking up into small, round, clear-cut masses ("chromolysis"). There are usually 3 to 6 of these, measuring 2 to 2.5 or 3 microns in diameter, though when but two are formed they may be 3 to 4 microns. Once liberated by breaking down of the cytoplasm, they are no longer identifiable as of leucocytic origin.

*Sections.*—Too little tissue has been examined to establish a typical histology. The lesion is primarily a chronic inflammatory infiltration of the chorium, with lymphoid and, more prominently, plasma cell accumulation. Eosinophiles are usually present and sometimes numerous. Abnormal accumulation of the normal pigmented cells of the chorium is sometimes seen.

Proliferative changes are sometimes evidenced by numerous prominent blood vessels, of small lumen but thick walls of prominent cells rich in protoplasm; these lie in a cellular connective tissue. Endothelial proliferation may diminish the lumen (figs. 9 and 11), sometimes obliterating it entirely and producing an area of endothelial cells that may suggest an early tubercle (figs. 10 and 12). In two areas of one section there were found a few giant cells with nuclei circumferentially arranged (fig. 17). As a whole, this process undoubtedly tends to make the tissue liable to degenerative changes. Acute inflammation, with infiltration and subsequent necrosis, apparently develops secondarily, though the causative agent is not evident. It may be seen developing in and about small vessels, in which thrombosis may occur (fig. 13), dependent upon inflammatory injury of the vessel, as in figs. 14 and 15.

The epidermis is usually somewhat hypertrophic. In one instance (fig. 7) it is peculiarly canalized by the papillæ, which sometimes extend very close to the surface. Pigmentation is diminished where hypertrophy is evident. The epidermis is not invaded, even at the edge of the ulcer. Loss of its integrity seems to be dependent on nutritional disturbances caused by the underlying lesion (fig. 8).

Few organisms of any sort can be found in appropriately stained sections, even in the acutely inflammatory areas, and none can be found in the deeper zones. That the acute reaction always results from secondary bacterial invasion seems, from this and the number of bacterial colonies that appeared in the

cultures, an inadequate explanation. There are sometimes found areas containing numerous minute to coccoid or larger granules and prominent shreds that stain intensely with basic dyes, as if of nuclear material (figs. 13, 14, and 15). Their source is not evident, unless they result from chromolysis of leucocytes, yet they are found in areas too recently invaded for necrosis of the leucocytes ordinarily to be expected.

In most sections nuclear changes in the leucocytes are noticeable. Among typically stained lymphocytes there may be more or less numerous round, solid, intensely staining bodies of the same size, apparently pycnotic individuals. In the acute exudate, both in the recently invaded areas and in regions of extensive infiltration and histolysis (figs. 18 and 19), polymorphonuclear leucocytes undergo, instead of ordinary necrosis, globular fragmentation (figs. 20 to 28, inclusive), by which the nuclei separate into 3 to 6 or 8 discrete, round, dense bodies, which lie in well-outlined pale red protoplasm. The size generally varies inversely with the number; occasionally there are as many as 12 to 15 fragments that are mere granules. This is evidently the same process as seen in the smears. The masses apparently do not long survive as such after the protoplasm breaks down, for free bodies are nowhere numerous.

In some cells there are but two bodies (figs. 20 and 27), and in others but a single, round, comparatively large nuclear mass. In its size and in the appearance of its protoplasm this mononuclear cell is similar to the multinucleated cells; it appears to be a polymorphonuclear the nucleus of which has fused into a solid, round mass.

#### CLINICAL DIFFERENTIATION

In certain clinical features, but especially in the impossibility of establishing a definite clinical diagnosis, this ulcer is like certain of those reported by previous authors. Leprosy, tuberculosis, discomycosis ("streptothricosis"), and "blastomycosis" of any type may be ruled out on the absence of the specific organism, as is dermal leishmaniosis, or oriental sore, which is not known to exist in these Islands. The lesion is clinically unlike the syphilitic ulcer, and the patients denied having had primary or secondary lesions.

The two lesions that have particularly to be differentiated are the tropical ulcer and late yaws. The former is rather the more similar and is not positively differentiable on clinical grounds, though those cases of tropical ulcer that I have seen usually did not exhibit the undermining so prominent in the



lesions under consideration. Neither the spirochæte of tropical ulcer nor the fusiform bacillus usually associated with it could be found in smears from these ulcers.

Tertiary yaws is, at least in the oriental tropics, very apt to be invoked in explanation of such lesions as the more advanced of these cases, and it is very probable that in yaws districts similar lesions, because of their negative microscopic findings and the beneficial effect of treatments that include potassium iodide, might be so diagnosed. I am strongly of the opinion that late yaws can be eliminated in the present instance because of dissimilarity of its development, course, and general appearance; negative history and absence of evidence of early yaws in these cases; and freedom of the immediate district from the disease. Negative search for the treponema in the chronic cases is not valid evidence in this consideration.

It is, therefore, probable that these lesions were due to some unknown infective agent, not demonstrable in smears or sections, but characterized by persistence in strictly localized lesions over long periods, and capability of causing considerable damage to the individual. This being borne in mind, the curious features of the cultural findings can be accepted as of more significance than might at first seem possible.

#### CULTURAL OBSERVATIONS

Utilizing tissue fragments and scrapings from incisions, a large number of cultures were made. The media used included ordinary nutrient agar, with and without dextrose, maltose, and glycerin, and a number of special fruit media. On none of the ordinary media and in no culture planted with fluid material were developments of interest observed. In several cultures mold fungi developed, evidently from spores contaminating the lesions. Bacterial contamination was practically constant, though in cultures of material from deep in the lesions there were few colonies. In most cultures the tissue elements were rapidly destroyed, as was to be expected from the combination of autolysis and bacterial overgrowth. However, in tissue planted on certain of the special media there occurred, whether in spite of, or dependent upon, the influence of the accompanying bacteria, certain changes in the leucocytes that are distinctly unusual and of considerable interest.

#### HYALINIZATION IN NUCLEI OF LEUCOCYTES

The term hyalinization is used as descriptive of the appearance in fresh preparations of the leucocytes whose nuclei, wholly

or in part, became firmer, larger, and more glistening and that separated, sometimes as if by fracture (fig. 31), into two or three distinct masses or fragments. In stained smears these masses usually showed well-defined outlines, with wide variation in color. The more advanced were oval or rounded and deeply stained. They were more or less differentiated from those nuclei, or parts of nuclei, that were not so intensified (figs. 29 to 38) and that sooner or later degenerated. Certain cultures in which bacteria did not multiply seemed unfavorable for this nuclear change, though it was apparently attempted (figs. 41, 42, and 43).

#### BASIC FORMS

These bodies are round, oval, or lenticular, sharply outlined, solid, intensely stained by Loeffler's blue and deeply stained by Giemsa's, without any determinable structural differentiation, and range in size from 2 to 4 or 4.5 or from 3.5 or 4 to 5 or 6 microns in different cultures (figs. 39 and 45). Because of their essential resemblance to the bodies to which I applied the term "basic form,"<sup>a</sup> they will be spoken of by that name. They were found only in cultures of tissue fragments on the fruit media and were never numerous. Bacterial growth seemed to facilitate their development, if not to be essential.

Their appearance in fresh preparations and stained smears indicate that they develop from the more deeply staining of the nuclear elements just described. They are often by no means sharply differentiated, for in a single preparation the gradation from pale degenerate cell fragments to intensely stained basic forms is not abrupt, though the range is usually wide.

The ultimate end of the basic forms is not evident from their appearance. Though in certain instances there was a strong suggestion of multiplication by fission, this cannot be asserted to have occurred. It is certain that their production was neither active nor sustained, and they more or less slowly disappeared.

#### CRYPTOCOCCUS

In one culture from each of cases I and II, there were found, after seven months and one and a half months, respectively, definitely differentiated fungous organisms, unicellular, of somewhat yeastlike morphology. These elements were small, usually measuring between 1.3 by 1.6 and 2.0 by 2.5, rarely 3.0, microns. They were sharply outlined, never doubly contoured, but often surrounded by an unstained halo, probably due to shrinkage

<sup>a</sup> *This Journal, Sec. B* (1916), 11, 267.

rather than to a special capsular structure. Wide variation in density of staining was often apparent. Young individuals (and particularly those in case I) tended to stain solidly (figs. 51 and 52). Older organisms (and particularly those in case II) were often granular (figs. 54, 55, and 56), some granules staining red by Giemsa's. In many groups some were unstained (fig. 59), appearing in stained, wet-mounted preparations as colorless hyaline bodies.

Multiplication took place by a peculiarly clumsy gemmation that sometimes approached binary fission (figs. 54, 55, and 57). It was evidently a very slow process, for though many individuals exhibited it, the numbers of cells produced were not great. Microscopically masses of them were found (fig. 49), but nothing approaching visible colonies was ever attained.

Cultivability was so low that in no transplant, even on the media ordinarily most favorable to fungus growth, was any development observed. It seemed as if the remnants of tissue elements were essential to their multiplication. When this was dispersed on fresh media, or exhausted in the original cultures, growth ceased, and the organisms did not long survive. It is possible that, together with special suitability of the medium (nutrient prune agar), symbiosis with the accompanying bacteria also may have had an influencing rôle in their development.

#### DETAILS OF CASES AND CULTIVATION WORK

In these reports the common descriptions, including the findings in smears and in sections of tissue fragments where such were examined, will not be detailed.

#### CASE I

*"Mycetomalike" foot with ulcers.*—M., Filipino rice farmer, aged 56 years, from a barrio somewhat distant from Hagonoy, seen August 17, 1916. The condition began with a painless soft tumor on the dorsum of the left foot two years ago. This did not ulcerate at first, but extended in the deeper tissues, the skin later breaking down and producing an ulcer that slowly enlarged, finally healing for the most part. When seen, the foot and ankle were thick and heavy, with marked induration extending from just behind the toes to above the ankle. The whole was rather suggestive of a mycetoma, except that there were no sinuses or discernible subcutaneous foci of necrosis. No bone destruction was evident. An area of skin over the dorsum was ulcerated, with scar tissue about it. Above this, on the ankle, was a large fungating ulcer (6 to 8 centimeters in diameter) said to be of about one year's standing. The floor was made up of soft pale granulations, from which material was removed for cultures and sections. I was unable to see this patient again, but the Commission surgeon reported that under potassium iodide the active lesions subsided completely.



Small pieces of tissue from three points in the lesion, superficial and deep, were planted (August 17) on nutrient banana agar and on banana cylinders. In smears (October 4) from the material on the latter, bodies of the cell-fragment and basic types were particularly noted, though they were at that time considered insignificant elements of tissue degeneration.

No further development having occurred, on November 18 (three months after planting) the deep-tissue fragment on banana cylinder was removed, crushed in a drop or two of sterile bouillon, and distributed to eight slants of special media. These included nutrient prune and nutrient banana agars (some with maltose added) and ordinary 2 per cent maltose and dextrose agars. The last two cultures quickly became overgrown with bacteria, among which very few cell fragments could be found; they were finally discarded as negative (January 3, 1917). In the fruit agar cultures (November 24) numbers of the forms were found, basic forms among them, particularly in the two on nutrient prune. On January 3 they had further increased in these, but were less numerous in the others. The material from several tubes was then transferred to tubes of plain (nonnutrient) banana agar slants, the parent tubes being kept for further observation.

In the subculture from maltose nutrient prune the various forms were numerous on January 17, and at several points in the smear there was a distinct grouping of the basic forms, as if they had multiplied in a mass. The other subplants showed few forms of interest. All were now sealed with paraffin and put away at body temperature.

On March 11 the smears from parent tubes (from which the November 24 transplants had been made) showed occasional small, round, basic forms on maltose nutrient prune, a very few apparently dividing by an indistinct process of fission. There were a few rounded and oval basic forms and numbers of the less definite cell-fragment bodies on nutrient banana.

In one nutrient prune agar culture there was found, among the staphylococci and fairly numerous cell-fragment and basic forms, a *Cryptococcus* essentially similar to that previously found in a culture in case II. In the smear it occurred singly and in small groups (figs. 51 and 52), though sometimes in larger masses (fig. 49). Nothing approaching colony formation could be detected.

The January 3 subplants now showed less numerous forms of interest than when last examined. Subplants were made from the two most promising of them, and a guinea pig (G. P. 73)

was inoculated intradermally. Transplants were also made from the *Cryptococcus* culture to several serum-containing agars. No evidence of further development was observed. On May 28 there remained insufficient *Cryptococcus*-containing material for further study, and all tubes were discarded.

#### CASE II

*Ulcers and scars of hands and wrist*.—K. M., Filipina, age 20 years, a laundress, seen August 17, 1916. On the back of the left hand was an old depressed scar from an ulcer that had begun two years before and persisted for several months, and in the palm of the right hand (fig. 2) was the scar of an ulcer that healed about a year previously. The active lesions were on the right hand and wrist; dorsally there was an irregular, apparently conglomerate ulcer extending to the fingers, of about one year's duration, apparently involving the deeper structures, the second and third fingers being maintained, though not rigidly, in flexion. Anteriorly on the wrist was another active ulcer, apparently confined to the superficial tissues.

On November 17 the dorsal ulcer (fig. 3) showed little change. The wrist lesion (fig. 2), however, was extending rapidly; above it was a new lesion, but a month old, that was typical, with undermining of the skin at the advancing border, an irregular, granular, very vascular base, and scar formation where the activity had subsided.

A fragment of tissue excised on August 3 from the wrist ulcer (lower) was planted on a banana-pulp cylinder. It proved to be badly contaminated. On November 17 tissue fragments and scrapings from superficial and deep levels of the newly developed, active ulcer were planted on various media (culture series 1 and 2).

*Culture series 1*.—This series included six cultures on nutrient banana and nutrient prune agars planted with small tissue fragments. On November 25 two were heavily overgrown with molds and bacteria and two others contained little but bacteria; these were discarded. A smear from a deep-tissue fragment on nutrient banana agar contained numerous small masses and bodies ranging from very pale indefinite fragments to clear-cut, densely stained bodies. Many similar forms were found on nutrient prune agar. The material from the nutrient banana culture was removed and distributed to culture series 3 and to fresh nutrient banana and prune slants; the latter were made anaërobic.

*Culture series 2*.—These cultures, planted simultaneously with series 1 with triturated tissue from deep in the lesion, included, among others, one slant each of: (1) Plain and (2) maltose nonnutrient prune, (3) plain and (4) maltose nutrient prune, (5) plain and (6) maltose nonnutrient banana, and (7) plain and (8) maltose nutrient banana agars.

In most of the cultures bacterial colonies appeared, though they developed poorly on the nonnutrient prune and banana agars. In smears from several cultures there were found occasional forms more or less approaching the basic type among the bacteria and irregular cell fragments, which were at first rather numerous. On December 17 they were still numerous on several media, though generally diminished. On January 3, 1917, the above described *Cryptococcus* was found in the culture on nutrient prune agar (medium 3). This was the first time that it had been observed. On medium 4 there were few of the cell-fragment forms, on 5 and 6 numbers of them were found, and on 1, 2, and 7 they were fairly numerous in the heavy bacterial growth.

The *Cryptococcus* was more plentiful on February 6, though nowhere was there a suggestion of colony formation. Various cell-fragment forms were still present in most of the other cultures. In culture 6 they seemed to have increased in numbers. In culture 4 some appeared to be attempting to differentiate into the *Cryptococcus*; this, however, did not materialize.

On March 11 the various formed elements having distinctly diminished, transplants were made to other special media, and material containing small numbers of the *Cryptococcus* was inoculated into a guinea pig (G. P. 44) intradermally and a rabbit (R. 43) intraperitoneally in a porous capsule. The results were negative.

*Culture series 3.*—On November 25 the material from a nutrient banana culture (culture series 1) was distributed on the same media as in series 2. The results are of interest only in that, starting with a uniform suspension, the forms here practically disappeared except on media 1 and 5, where they seemed to multiply temporarily. The anaërobic cultures were negative.

#### CASE III

*Multiple ulcers of right leg and foot.*—L. A., Filipina, age 14, seen August 17, 1916. Occupation, housework; seldom in fields. The primary lesion had appeared about a year previously on the right small toe (fig. 5), which had become thickened, decidedly shortened, and was superficially ulcerated. The evident involvement of the deeper tissues, with but a very shallow ulcer, was noteworthy. The consistence of the exposed tissue could not be investigated, the lesion being very sensitive.

On the inner surface of the leg were two groups of ulcers more or less separated by normal skin and scar tissue. The first had appeared seven months previously. Anteriorly on the ankle was an abruptly raised lesion 3 to 4 centimeters in diameter. The skin at the edge was not indurated, but was turned abruptly outward, as if by a draw string, for about 0.8 centimeter. The plateau thus produced consisted of granular friable in-



flammatory tissue. On the inner surface of the foot was a firm swelling 3 by 5 centimeters, evidently a developing focus, covered by intact skin; this was of several months' duration. Material for cultures and smears was removed from open ulcers, but incision of the unopened mass was not permitted.

On November 18 the lesion over the ankle had lost its fungating character (figs. 4 and 5), the mass on the inside of the foot was represented by irregular ulcers, and those on the leg (fig. 4) had enlarged and were more fused. The condition, as a whole, seemed more active. Small fragments of superficial tissue were again secured.

On August 17 material from three different lesions was planted on nutrient banana agar and banana cylinders. The cultures became overgrown with bacteria—*B. pyocyaneus* and *Staphylococcus aureus* chiefly—and molds and were discarded.

On November 17 other tissue fragments were obtained from two active ulcers, though not sufficiently deep for satisfactory cultivation work, and plants were made on various media. On November 25 numbers of forms approaching the basic were found in several cultures, few in others. On January 2, 1917, there was apparently no increase of these bodies where they were to be found. From three of the possibly favorable cultures the material was transferred to plain banana agar slants. On February 10 a very few basic forms were found in one of the subcultures. Subplants from these to special media, and a guinea pig (G. P. 110) inoculated intradermally, gave no results.

*Comment.*—Though the lesions in this case were fairly active, satisfactory material was not obtained. The lesions seemed very sensitive, and the patient was unusually timorous. Being ignorant and careless, she had neglected the lesions, and contaminating organisms were particularly numerous.

#### CASE IV

*Chronic ulcers of left leg, with deep scars.*—The patient, T. P., a Filipino, farmer, aged 19 years, was seen November 17, 1916. He was somewhat undersized and not robust. The condition had begun eight years before as a small sore at the knee. This had spread, evidently superficially, downward over the entire inner surface and much of the outer, leaving a soft, thin, parchmentlike skin extending to the inner malleolus.

On the inner surface of the lower half of the leg were three irregular ulcers (two shown in fig. 6) in an area of marked tissue loss. This is poorly seen from the angle at which the photograph was taken. Fibrosis was extensive and involved the muscular tissue, with some limitation of motion.

The ulcers were fairly typical, extending outward in the more or less fibrotic tissue, with slow undermining of the epidermis (fig. 8) and induration of the overlying layer. Healing occurred centrally as the lesions progressed outwardly, leaving scar tissue (fig. 6). These lesions were said to have been active for two years, but it could not be determined positively

whether this was a new infection on the superficial scar of the original lesion or whether it was a renewal and extension of the latter. The progress of the infection was very slow; the Commission surgeon reported that the lesion was practically unchanged in three months.

Tissue for cultures and sections was removed from the edges of the most active-looking lesion, and smears were made from several points. These show no special feature on microscopic examination.

Two tissue bits from deep in the lesion were each divided and planted on nutrient banana and nutrient prune agars. Bacterial growths promptly appeared in all. On November 24 smears of the softened tissue on nutrient banana showed, among the staphylococci, many cell fragments and densely staining forms. Very few of the latter were found. In transplants from some of these cultures nothing but bacterial growth developed, and they were finally discarded.

After six weeks on one of the original nutrient prune cultures the fragment of tissue was removed (January 2) and triturated. Occasional clear-cut basic forms and numerous more or less fragmented cells, numbers of them deeply staining, were found. Subplants of this material were made on plain and nutrient banana agars. A rabbit (R. 64) was inoculated in the anterior chamber; it died in three days, apparently from staphylococcic invasion. On January 17 the plain banana subplant showed numbers of small, usually round, basic forms. The nutrient banana subplant was discarded because of contamination. No change occurring, further subplants to several other special media were made (March 13), another rabbit (R. 108) was inoculated, and two or three drops of sterile serum were added to the original culture. No further development occurred, and on June 28 all tubes were discarded. This rabbit remained negative.

*Comment.*—This case presents the lesions of longest standing in the series, the greatest area of involvement, and the most serious tissue loss. The lesions were very indolent and, therefore, presumably not rich in the infecting agent.

#### CASE V

*Early lesion, right foot.*—B. S., a Filipino, 25 years of age, farmer, in excellent general condition, was seen on November 17, 1916. Six weeks previously a small pustule had appeared at the center of the lesion presented (fig. 1). This had been scratched and frequently irritated and had slowly enlarged.

When seen, the lesion appeared as an area of superficial erosion without ulceration, measuring 4 by 5 centimeters, on and behind the inner malleolus. This area was rough, pale superficially, red in the deeper portions. At about the center (arrow, fig. 1) there was a slight opening that led into a flat, collapsed cavity. A probe inserted obliquely passed under the skin

for about 1 centimeter downward, forward, or upward. The process had confined itself to the subcutaneous tissue. Upon incising the overlying skin, the cavity was found to be lined by a thin layer of pale necrotic material over vascular inflammatory tissue.

Because of its situation no tissue was removed from the floor, and only a small piece was removed from the skin itself. Smears showed bacteria, but nothing else of interest. The tissue section is shown in fig. 7.

Small bits of tissue were planted on nutrient banana and nutrient prune agars. On November 25 both tubes showed bacterial growth, more on the banana than the prune. In smears from the former none of the cell-fragment forms were found, though a few were present on January 2, at which time the material was transplanted to plain and fresh nutrient banana agars. On January 17, these cultures being negative, they were sealed with paraffin and put aside at body temperature. On February 20 they were still negative and were discarded.

The nutrient prune culture had shown (November 25) a few suggestive forms. Transplants were made (January 2) from this to plain and (fresh) nutrient banana agars. On January 17 the nutrient banana subcultures showed heavy staphylococcus growth and practically no forms of interest. On the plain banana, however, on which the bacteria were largely inhibited, numbers of cell fragments were found. All tubes were paraffined and put away at body temperature. On February 20 only the two plain banana cultures showed a few cell-fragment forms; a few were still to be found on March 16, but attempts to stimulate development were unsuccessful. A rabbit (R. 111) was inoculated in the anterior chamber without result.

*Comment.*—This, the earliest case in the series, I found accidentally in the clinic as it was being dressed as an insignificant lesion by the clinic attendant. So far as the preliminary findings indicate, it is probably of the same nature as the above cases.

#### CASE VI

*Inflammatory tumor beneath scalp.*—The patient, Filipina, aged about 30 years, complained, when seen August 3, 1916, of a painful tumor of the size of a small olive beneath the scalp on the left side. This had troubled her increasingly for three weeks, finally preventing sleep. The mass was adherent to the bone, not to the scalp, and was painful, firm, not fluctuant. The patient presented no other lesions.

Exploration by needle elicited no evidence of necrosis or suppuration. Incision was not permitted. By aspiration two or three drops of bloody fluid were obtained. In a single culture on dextrose agar this was negative. Microscopically no organism was found. High lymphocyte and eosinophile content (above 10 per cent) suggested a chronic inflammatory condition.



Subsequently the mass was incised by the Commission surgeon, who found only well-vascularized tissue, apparently inflammatory. The condition not being relieved, he then infiltrated the mass with 95 per cent alcohol, 5 cubic centimeters, twice, two days apart. This caused acute pain, which slowly subsided, ceasing entirely after a few days, when the mass began to diminish. When the patient was next seen by me (November 17), there was no trace of the mass itself, but a distinct irregular, shallow area of depression, about 1 by 2 centimeters, could be felt in the bone. This evidently resulted from erosion by the tumor, and probably explains the severe pain.

*Comment.*—The etiology of this lesion is, of course, unknown. The efficacy of the treatment applied bespeaks an inflammatory process. The findings in the aspirated material (few polymorphonuclears and no organisms) and a negative culture are against a bacterial cause. The history, in ulcer cases, of similar nodules preceding ulceration, the apparent lymphocytic and eosinophilic infiltration, and the curious tendency to affect the bone, corresponding to deep invasion in some of the ulcer cases, makes this lesion of interest as possibly of the same nature.

#### SUMMARY AND DISCUSSION

In the cases detailed above, search along usual lines for a demonstrable causal organism of recognized type, whether bacterial, protozoal, or fungous, has been totally without result. The lesions, which present clinical evidence of etiological similarity, are clearly not bacterial, leishmanial, or of the ordinary tropical phagedenic type. There is no positive evidence that it is of syphilitic or frambœsial origin, nor is a treponema demonstrable in scrapings. Clinically, therefore, it seems to fall into the category of ulcerative and destructive processes of unknown etiology.

Intensive cultural study of excised tissues has resulted in observations that are suggestive of a hitherto unrecognized process of infection. On ordinary media bacterial overgrowth was always prompt and the tissue elements rapidly degenerated; no special feature was seen. On special media, however (nutrient banana and, particularly, nutrient prune agars), leucocytes of the planted tissue fragments instead of disintegrating underwent, in spite of the combined influence of autolysis and bacterial overgrowth, a change unlike that ordinarily to be seen in such material. This was a peculiar solidification (suggesting hyalinization) that the nuclei underwent, whereby they or parts of them became more deeply staining. This is no doubt in part due to the peculiar effect of these media, on which tissue autolysis is often considerably inhibited. However, apparently by further

changes in the denser of these nuclear fragments there developed bodies that are, I believe, not referable to changes induced by the media alone. These were firm, oval or round bodies, deeply staining by Loeffler's methylene blue and similar stains, clear-cut in outline, an appearance that suggested parasitic fungous elements.

In one culture only from each of two cases of the series there was finally found a definitely fungous element, a *Cryptococcus*.<sup>6</sup> In one case it was found after cultivation for six weeks on nutrient prune agar, but in the other only after seven and a half months on culture media, three months on a banana cylinder, and four and a half months on nutrient prune agar.

The source of the *Cryptococcus* is not clear. No similar organism is to be found in earlier smears from those cultures in which it developed. Difficult as it may be to accept, there seems to be a direct parental relationship on the part of the described basic bodies. Certainly it is not possible to differentiate between some of the typical basic forms and some of the more intensely stained fungus cells.

The significance of the fungus depends, of course, upon its source. On the evidence it cannot be proved to be essential in the lesion, though the conditions under which it developed led me to believe this to be the case. To establish this as a fact, cultivation of the organism in sufficient amount to permit the production of experimental lesions with it would be necessary. Though the material available, in large part bacterial growth, was expended entirely in attempts at subcultivation, this was not accomplished. Animal experimentation could, therefore, not be attempted.

To deny this organism an essential rôle, one must assume it to be a contaminant, either of the lesions or of the two cultures involved. If it was in the original material as removed from the lesion, it certainly was not evident at the time. Furthermore, had this been the case, it or some recognizable precursor should have appeared earlier in those cultures containing it and in more of the cultures made from the tissue fragments in those cases.

<sup>6</sup> The genus *Cryptococcus* Kütz. 1843 includes organisms that reproduce by budding only; they do not produce mycelia, or spores. In this group fall, in default of essential information, the degraded yeastlike forms that may be adopted, with more or less permanency, by higher fungi of widely diverse classification.

The possibility that it is a laboratory contaminant of the cultures cannot, of course, be denied. However, in view of its characteristics, and in the light of experience with the conditions in this laboratory, this seems to me improbable. The cultures were paraffin-sealed, and at no time showed any evidence whatever of ordinary contamination. They at first contained only the curious cell-fragment forms among the bacteria, a staphylococcus in one case and a proteuslike bacillus in the other. After different periods of time the *Cryptococcus* was also found.

The fungus itself was unlike any of the many strains of yeast-like organisms of which I have found descriptions or have myself met with. I have never seen any laboratory contaminant that resembled it in the slightest, and it is totally unlike the fungi of that class that I have from time to time cultivated from sputum, urine, fæces, fruit, etc., here and elsewhere. Its characteristic features of nonproduction of visible colonies, of an unusual and evidently very slow process of multiplication, of noncultivability in fresh subplants away from the remnant of the tissue material originally planted, though put on media particularly adapted to the cultivation of fungi, are not suggestive of a saprophyte but, on the contrary, bespeak a highly parasitic organism.

The alternative that the observed fungus is essential to the lesion seems, therefore, quite possible. The appearance of the lesion itself and its course and reaction to potassium iodide are indicative of mycotic infection. Though no fungus element can be demonstrated in preparations from it, the same is true of other microorganisms. The fungus that was found appeared only in cultures of material from two clinically active cases, on a medium (nutrient prune agar) that is especially favorable to the growth of fungi. The characteristics of the two strains seem to be identical and are essentially those of a highly parasitic organism, as might be expected of the causal agent of such lesions. Therefore the weight of evidence, positive and negative, justifies the serious contemplation, if not the assumption, of the conclusion that the described fungus element represented the infecting organism.

From the mycologist's point of view the *Cryptococcus* is, undoubtedly, to be regarded as a degraded form of a higher fungus. From the viewpoint of the hypothesis outlined above, however, assuming that it is the causal organism, it is apparent that it may



actually be a comparatively highly differentiated phase of an organism not to be demonstrated morphologically in the lesion to which it gives rise.

In what form such an organism would maintain itself in the tissues is not evident from the findings. If it is as a formed body, it probably resembles so closely some tissue cell as to be indistinguishable morphologically or tinctorially. The alternative is that it occurs as a body or substance with little or no morphological differentiation, whether maintained intra- or extra-cellularly. The observed development of the peculiar basic forms from tissue elements in cultures, in conjunction with certain of the findings in the lesion, suggests the existence of an amorphous intracellular substance that is passed on from cell to cell by disintegration of those infected. The granules and shreds seen in some of the tissue sections (as in figs. 13, 14, and 15), and the peculiarly orderly fragmentation of degenerating leucocyte nuclei sometimes seen in older areas (figs. 18 to 28), might play a part in such a process. Such a substance would be, in effect, a localized, nondiffusible "virus" and would perhaps be midway between organisms of fixed morphology and the recognized filterable viruses that are known only by their constitutional effects.

The difficulty of determining whether or not the tissue or cultural cell fragments are of pathogenic significance, and not simply negligible degeneration products, is obvious. The fragmentation seen in sections, though interesting, is by accepted standards ascribable solely to pycnotic degeneration (chromolysis) and does not suggest any vital process. Further, in preparations from cultures none but the clear-cut basic forms are at all convincing as of possible significance; the lesser forms usually appear as negligible fragments of tissue cells that are often seen on the special media employed. However, the development of the denser forms and their persistence in spite of very unfavorable influences where developed distinguish them as of more than incidental importance.

The contagiousness of this infection is very slight or nil. No history of contact with previous cases or of subsequent infection of other individuals could be obtained. Were the exudate very infectious, this would be a simple matter because of intimacy and extreme hygienic simplicity of barrio life among this class of Filipinos. From the viewpoint of the suggested etiology of the lesion it may be that the invader, after adaptation between itself and the host became established, and while losing its mor-

phologic identity, also lost its ability to infect a fresh individual, or to return to a saprophytic, differentiated form. This seems entirely possible in view of the profound, sometimes essentially (ultimately) fatal, modifications that may be undergone by such organisms in adapting themselves to animal parasitism.

The hypothesis on which the suggested interpretation is based may seem fantastic. Nevertheless the observations described and those yet to be reported seem to permit of but one other possible explanation. This involves the recognition as pathogens of organisms belonging to the peculiar borderline group that includes the Myxomycetes or Mycetozoa, which are not recognized as of any importance whatever in animal pathology. For the present it will suffice to call attention to the possibility. The hypothesis adopted, based on modification of true fungi, seems at this time to be the more conservative and the more probable view. However, the distinction is not fundamental, for some of the lower fungi (such as the Chytridineæ) and the Myxomycetes are in some respects closely allied.

Of whatever type the invading organism may finally prove to be, the process of "cryptoplasmic" infection outlined is based primarily on observations and should be correct. At all events, there is presented a most baffling problem, one that is not easy to approach. Because of its essential peculiarities it will be very difficult definitely to establish it. On the other hand, since negative results are often inconclusive, it will be equally difficult positively to disprove it.

#### CONCLUSIONS

There has been found in one district of the Philippine Islands a type of lesion, invasive, and usually more prominently ulcerative, of undetermined etiology. On clinical grounds it seems very possibly mycotic. No parasite of any type, protozoal, bacterial, or fungous, can be demonstrated in the lesion. Fragmentation of the nuclei of polymorphonuclei into separate rounded masses ("chromolysis") is sometimes interestingly prominent.

In certain cultures on special media, of tissues excised from the lesion, the nuclear bodies of leucocytes have undergone modification to produce clear-cut, intensely staining "basic" forms. From each of two cases, in one culture only, there has developed a *Cryptococcus* of very low cultivability and of cultural requirements suggesting high parasiticity. This is thought not to be a contaminator but, in the absence of any recognizable precursor, possibly to be a development of the basic forms.

This suggested evolution can be explained only on the basis of the hypothesis that the infecting organism may have assumed a cryptoplasmic state on adapting itself to animal parasitism, followed by the invasion and utilization of the tissue cells, particularly the polymorphonuclears.

The general hypothesis as outlined has been formulated as a possible explanation of these and other more or less related observations, which it is the intention to report in the series of articles undertaken.



## ILLUSTRATIONS

### PLATE I

- FIG. 1. Early lesion, duration six weeks. Before ulceration. Considerable undermining of skin with but a small sinus. Case V.
2. Ulcers of wrist; upper lesion unusually active, duration one month; lower lesion several months. Scar in palm from previous lesion. Case II.
3. Spreading lesion on dorsum of same hand, same case. Duration about one year.
4. Lesions of leg, case III. Irregular ulcer on inner surface of foot marks the site of a smooth swelling, skin intact, seen three months previously.
5. Original lesion, case III. Involvement of bone with some shortening of toe. Duration about fifteen months.
6. Lesions of leg, case IV. Full extent of tissue loss not apparent at the angle shown. Duration at least two years.

### PLATE II

- FIG. 7. Section from advancing edge of leg ulcer, case IV (see fig. 6), showing hyperplasia and canalization of epidermis, with absence of infiltration.  $\times 11$ .
8. Similar section from case I. Thinning of epidermis at edge of ulcer evidently secondary to inflammatory lesion below.  $\times 12.5$ .
9. Endothelial proliferation in small blood vessel, without infiltration.  $\times 300$ .
10. Tuberclelike focus apparently marking site of small vessel.  $\times 300$ . (Figs. 9 and 10 from same section as fig. 7.)
11. Vessel in center shows both proliferation and early leucocytic infiltration.  $\times 180$ .
12. Focus of endothelial cells, with a few leucocytes, apparently resulting from obliteration of a vessel.  $\times 180$ .
13. Vessel on left contains red blood cells; lumen of that on right almost obliterated; that in center obliterated by a thrombus containing small, deeply stained granules and fragments.  $\times 180$ .
14. Area of small vessels with considerable leucocytic infiltration. Deeply stained granules (nuclear fragments?) and shreds are prominent.  $\times 180$ .

### PLATE III

- FIG. 15. Area somewhat similar to fig. 14.  $\times 300$ .
16. Large (mitotic endothelial?) cell seen in lower part of fig. 15.  $\times 650$ .
17. Group of six Langerhans's giant cells.  $\times 300$ .  
(Figs. 11 to 17, inclusive, from deep tissue, case III.)

- FIGS. 18 and 19. Exudate in superficial necrotic tissue from floor of ulcer. Normal-staining polymorphonuclear leucocytes contrasted to deeply staining and fragmenting cells.  $\times 500$ .
- FIG. 20. Contrasting cell containing two nuclear masses with ordinary, less densely stained polymorphonuclear.  $\times 950$ .
- FIGS. 21 and 22. Cells with three nuclear masses.  $\times 950$ .
- FIG. 23. Cell with six nuclear masses (one not visible in this plane).  $\times 950$ .
- FIGS. 24 and 25. Groups of small nuclear masses as deposited by breaking up of the cell.  $\times 950$ .
- FIG. 26. Large cell with six nuclear masses (one not seen), all distinctly crescentic (apparently degenerating).  $\times 950$ .
27. Two nuclear masses in a leucocyte, density of staining much greater than in surrounding cells.  $\times 950$ .
28. Two separate bodies, without protoplasm. Whether produced as in fig. 27 is not evident.  $\times 950$ .
- (Figs. 18 to 28, inclusive, from superficial tissue, case III.)

## PLATE IV

- FIGS. 29 and 30. Leucocytes separating into deeply staining lobules. Smear from culture, case IV, eight days on nutrient prune agar.  $\times 1,000$ .
- FIG. 31. Leucocyte dividing into three sharply edged fragments, of hyaline appearance.  $\times 1,000$ .
32. Leucocyte producing two rather deeply staining bodies.  $\times 1,000$ .
33. Leucocyte divided into three parts, one becoming oval and deeply staining, others soft, diffuse, pale-staining.  $\times 1,000$ .
34. Oval body in amorphous remnant of cell.  $\times 1,000$ .
- FIGS. 35 and 36. Soft, amorphous degenerating cells. Clear-cut, round bodies (approaching the basic form) developed elsewhere.  $\times 1,000$ .
- FIG. 37. Large body apparently degenerating.  $\times 1,000$ .
38. Free-lying body approaching basic.  $\times 1,000$ .
- (Figs. 31 to 38, inclusive, from smear from culture, case IV, eight days on nutrient banana agar.)
39. Typical sharply outlined, densely stained basic form. From case I, heavily contaminated culture on partially favorable medium.  $\times 840$ .
40. Irregular mass of degenerating cell material, quite unlike the basic forms. From case I, heavily contaminated culture on unfavorable medium.  $\times 840$ .
- FIGS. 41, 42, and 43. Leucocytes with deeply staining nuclear masses in smear from tissue fragment, case I, triturated after three months on banana cylinder. Bacterial growth slight.  $\times 840$ . This material transplanted to nutrient prune agar gave large basic forms; on other media it degenerated more or less promptly.
- FIG. 44. Two forms approaching basic, apparently derived from one cell.  $\times 840$ .
45. Clear-cut basic form.  $\times 840$ .
46. Irregular body (approaching basic type) and very small clear-cut body suggestive of an organism.  $\times 840$ .

## PLATE V

- FIGS. 47 and 48. Groups of more or less clear-cut, deeply stained cell fragments and basic forms.  $\times 840$ .  
(Figs. 44 to 48, inclusive, from case II, culture of tissue fragments eight days on nutrient prune agar.)
- FIG. 49. Masses of cryptococci, case I. Culture of tissue fragment three months on banana cylinder and four months on nutrient prune agar.  $\times 1,000$ .
50. Scattered cryptococci, case II. Culture of tissue fragments six weeks on nutrient prune agar.
- FIGS. 51 and 52. Small groups of the cryptococcus, case I, same smear as fig. 49. Showing density of staining and irregularity in size and outline.  $\times 1,150$ .
- FIG. 53. Cryptococci, case II, from same culture as fig. 50. Showing (arrow) apparent attempt at mycelial growth. (This offshoot in the smear is clearly distinct from the surrounding bacteria.)  $\times 1,300$ .
- FIGS. 54, 55, and 56. Showing irregularity of size, shape, and mode of multiplication, neither clear-cut budding nor simple binary fission.  $\times 1,150$ .
- FIG. 57. Group of four sharply pointed apple-pip-like cryptococci. Their shape and close aggregation suggest origin by fission in two planes.  $\times 1,150$ .
- FIGS. 58 and 59. Cryptococci apparently degenerating, becoming pale staining in the former and unstained in the latter. Spaces believed not to represent organisms dropped out of smears.  $\times 1,000$ .  
(Figs. 54 to 59, inclusive, from same smear as fig. 53.) (Cultural forms all stained with Loeffler's methylene blue.)



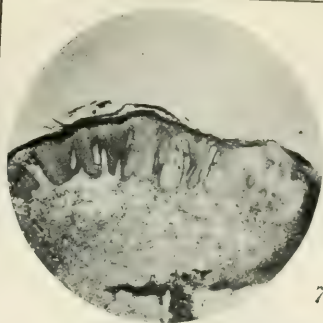




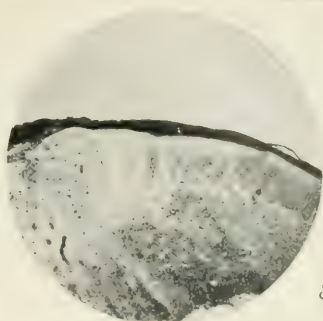
PLATE I.



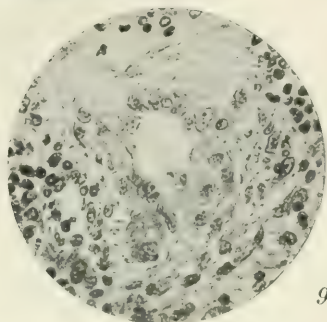




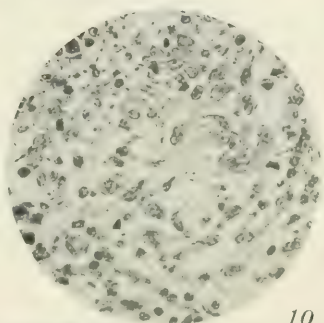
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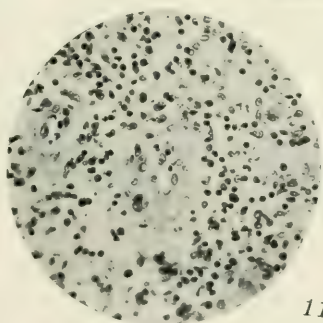
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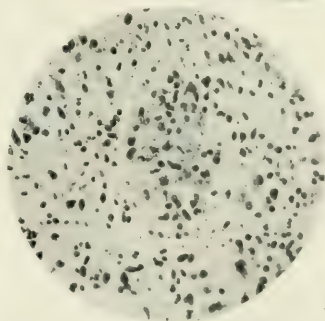
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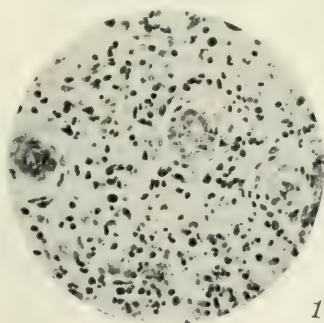
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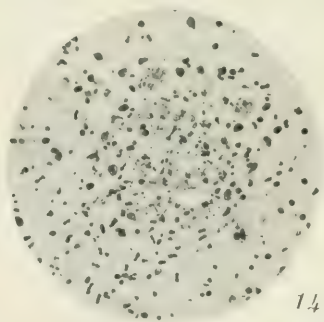
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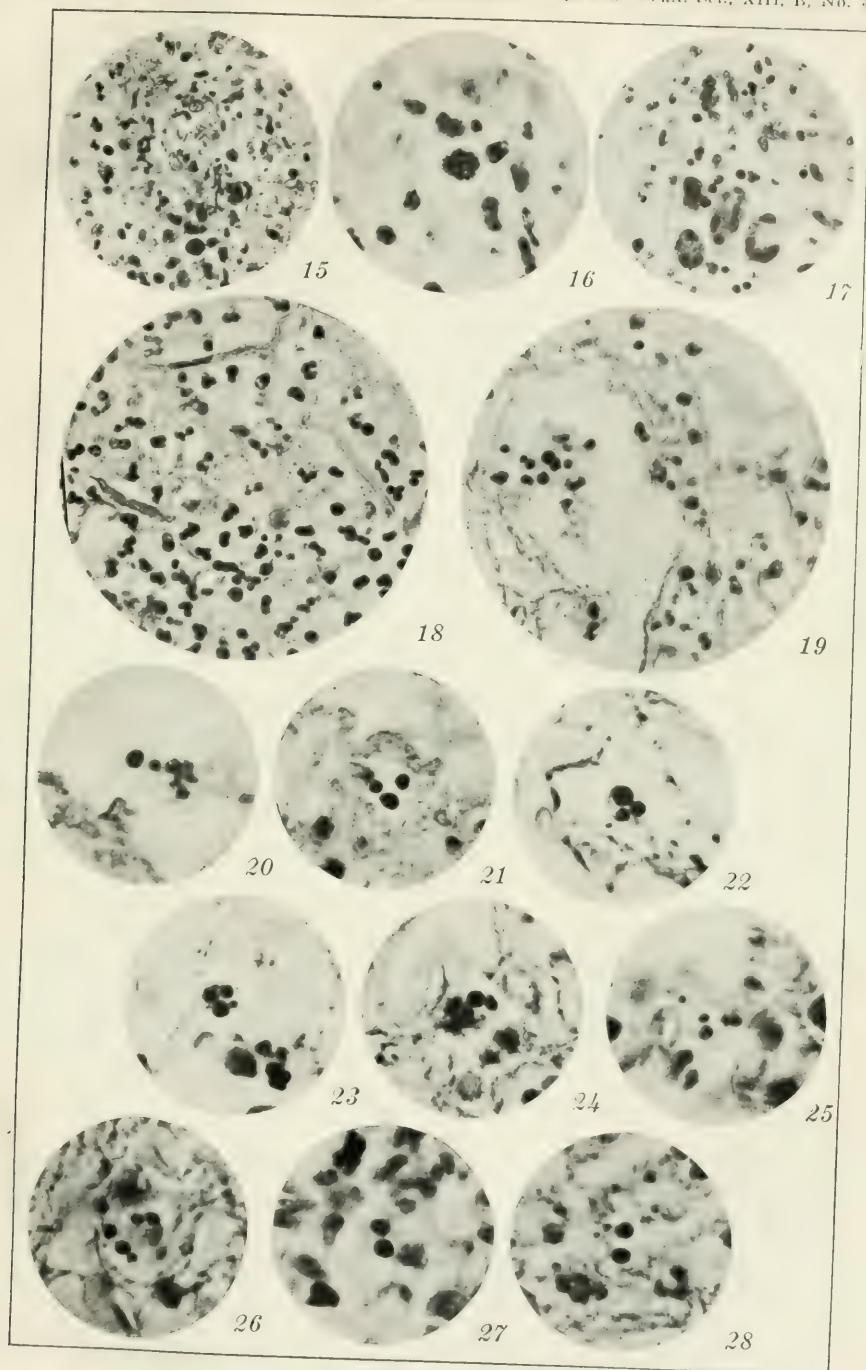


PLATE III.





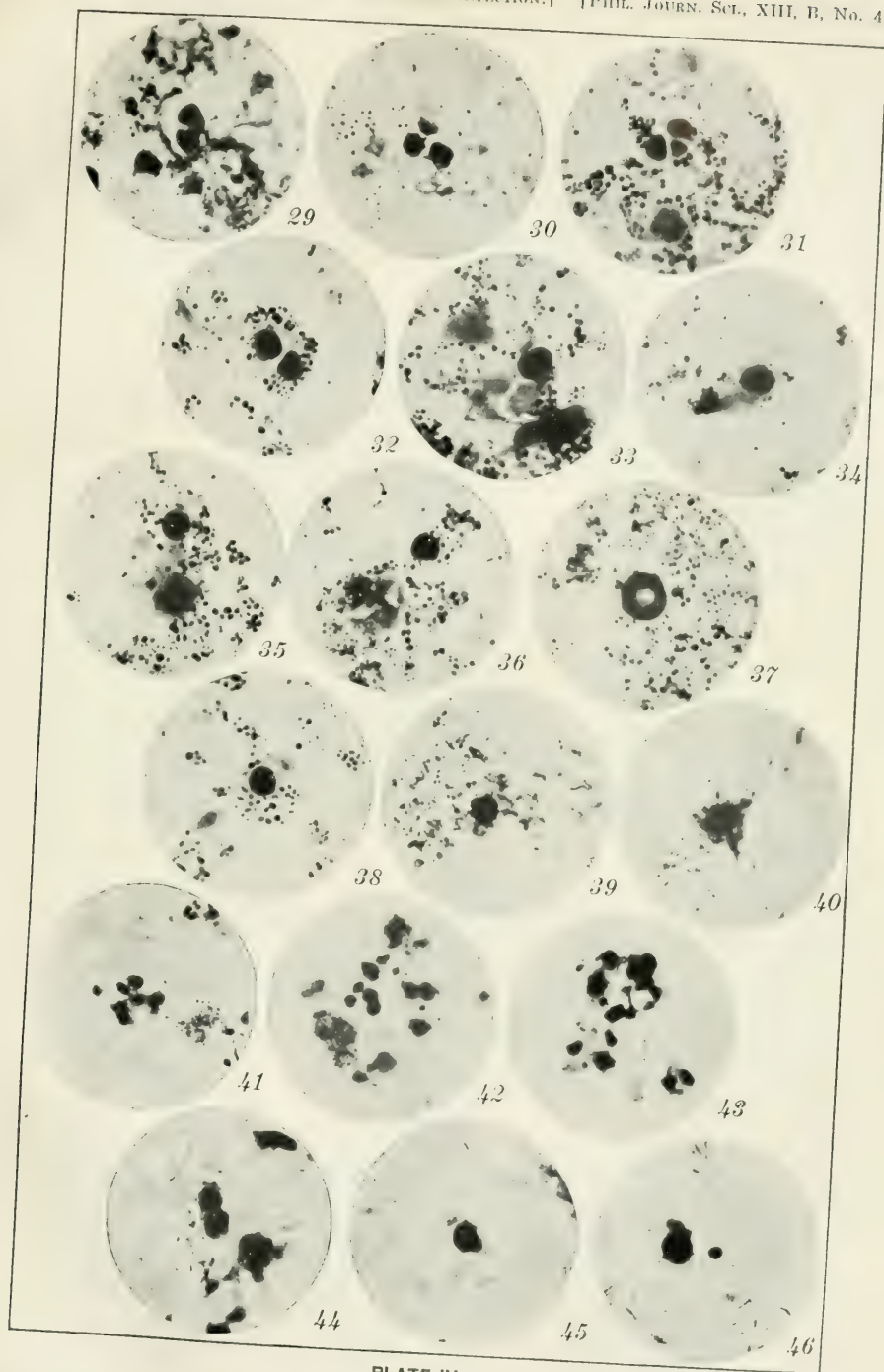


PLATE IV.





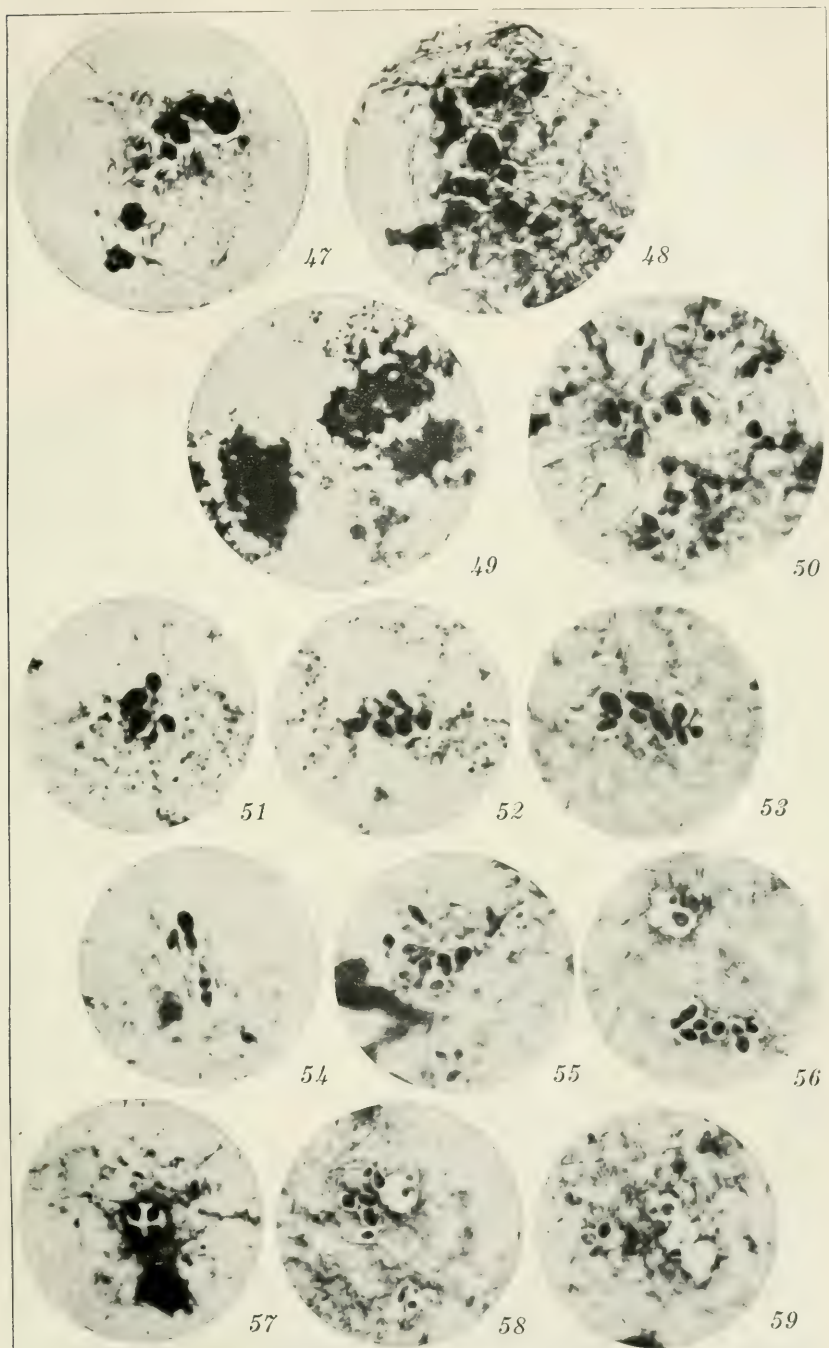


PLATE V.



## FURTHER OBSERVATIONS ON THE TREATMENT OF YAWS WITH CASTELLANI'S MIXTURE

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### TWO PLATES

Salvarsan and neosalvarsan are, without doubt, the specific remedies for frambœsia; but the high price and shortage of these drugs brought about by the war, the lack of hospital facilities in districts where the disease prevails, and the fact that patients often refuse any kind of injection treatment are some of the considerations that led Castellani, (1, 2) in 1915, in Ceylon, and us in the following experiments to use his formula in place of the drugs. The formula consists of:

	Quantity.
Tartar emetic.....grams.....	0.065
Sodium salicylate.....do.....	0.65
Potassium iodide.....do.....	4
Sodium bicarbonate.....do.....	1
Water.....do.....	30

The above is given in one dose, diluted in 4 ounces of water, thrice daily, for adults and for children over 14 years of age, half doses to children 8 to 14 years of age, one-third doses or less to younger children, and not more than half doses to Europeans. According to Castellani the preparations of antimony, which were first introduced in the treatment of yaws by Brault in 1911, have a beneficial effect, but their action is slow. Sodium salicylate apparently hastens the disappearance of the thick yellow crusts, due to secondary pyogenic infections. Potassium iodide potentiates tartar emetic, but its great drawback is that the good effects are obtained only by using doses that often give rise to iodism. This and the emetic action of the antimony and potassium tartrate are diminished by the addition of sodium bicarbonate. The formula is pharmaceutically a very inelegant one; it is cloudy and has a sediment due to the formation of antimony



oxide. The sediment, however, disappears when each dose is diluted with four times the amount of water or when 8 cubic centimeters of glycerin are added to the formula.

Castellani tried this formula in eleven cases given in the doses mentioned for from ten to fifteen days, followed by from five to ten days of rest, then another course for another five or ten or fifteen days. His results were very satisfactory in recent and fairly recent cases, in which the disease had started three to twelve months previously. In chronic cases the results were much less satisfactory. Very mild symptoms of iodism were observed in three cases, but were not sufficiently severe to necessitate stopping the treatment or decreasing the doses. No symptoms pointing to any depressing action on the heart were noted.

Spaar(5) obtained excellent results with this treatment in three cases, the patients recovering in two or three weeks.

We have used this treatment in more than 43 cases, including 7 in the Philippine General Hospital, 14 in Parañaque, and 22 in Las Piñas. The cases in the Philippine General Hospital were all discharged completely cured.

With the exception of four, all our cases presented, more or less moderately, one or several of the following symptoms during the treatment: General malaise, weakness, slight fever, nausea, vomiting, gastralgia, diarrhœa, pharyngitis, ptyalism, intense coryza, lachrymation, congestion of the conjunctiva, cephalalgia, and insomnia. One case also presented slight induration or nodules of the skin of the face and ears, and another presented erythematous patches in various parts of the body. However, we did not observe in those cases who were under our immediate supervision furunculosis, acne, purpura, nor œdema of the eyelids, face, and larynx. The method of administration followed by us brings about gradual saturation of the body. We gave on the first day one-third dose three times; on the second day, one dose twice; and on the subsequent days, one dose three times daily. We adopted this dosage in order to ascertain the susceptibility and gradually to establish tolerance in the patient. The number of doses necessary to effect a cure varied from 15 to 80, and the time elapsing from the commencement of the treatment till complete recovery was from five to twenty-seven days.

Of the 14 cases of Parañaque, 10 showed complete recovery, while the remaining 4 showed only improvement of the symptoms. Seven of the Las Piñas cases discontinued the treatment early. 3 showed improvement of symptoms, 3 proved refractory, and 9 recovered completely. To summarize: Of the 36 cases that continued the treatment, 24 completely recovered, 7 showed im-

provement of symptoms, 7 showed no improvement at all, and 5 had relapses in from two to five months after the lesions had entirely healed.

The details of the cases treated and the results of the treatment are given in the subjoined protocols:

*Case I.*—M. P., female, 45 years old, a native of Bulacan. The duration of the infection was about nine months. The lesions consisted mainly of granulomatous ulcers on the labia majora and a few papillomatous growths about the nasal fossæ and the left angle of the mouth. The growth was covered with a thick yellowish crust. The ulcerations on both labia excreted a purulent fetid material. The general appearance of the growth simulated "granuloma pudendi." The patient received 44 doses of Castellani's mixture in nineteen days, and sixteen days later the lesions were completely healed.

*Case II.*—F. P., female, 24 years old. The infection was of six months' duration. The lesions consisted of warty growths on the forehead, face, neck, abdomen, and genitals. The mother yaw was an ulcer with irregular borders, 2 centimeters in diameter, on the anterior surface of the left ankle. The patient entered the hospital to obtain relief from pain in the bones and joints. The joints of the fingers and hands were swollen and painful. She received 23 doses in ten days and left the hospital after fifteen days with complete disappearance of arthritic pain and cicatrization of the lesions.

*Case III.*—C. F., male, 30 years, married, denied having had venereal diseases. The duration of the infection was one year and four months. The patient thought that he had contracted the disease from his children, who had yaws. The primary lesion appeared as a bed bug bite on the left thigh. On scratching the skin over it, the lesion gradually increased in size to about 10 centimeters in diameter. Two months later eruptions appeared all over the body, but after a year they disappeared without treatment. Last January he was admitted to the hospital, complaining of pain in the bones. He presented extensive ulcerations on the soles of both feet. The Wassermann test was negative. The patient was given 15 doses in eleven days. He left the hospital completely cured.

*Case IV.*—E. Y., female, 19 years old, contracted yaws six months ago. She said she had had yaws when she was 12 years old. The scars were plainly visible on the face and on other parts of the body. She had an ulcer on the anterior aspect of the right leg, which measured 3 centimeters in diameter and 1 centimeter deep. The edges were irregular, and the base was formed by necrotic tissue and exposed bone. Wassermann reaction was positive. The patient was given 5 drops of saturated solution of potassium iodide and 5 milligrams of protoiodide of mercury three times daily. Aseptic dressing was applied to the ulcer. She experienced no improvement from this treatment. Castellani's formula was then given three times daily, and 25 per cent protargol ointment was applied locally. After 54 doses the ulcer completely healed, although the pain in the bones persisted for some time.

*Case V.*—M. Santa Ana, male, 38 years old. The duration of the illness was five months. The lesions consisted of granulomatous growths of varying size, whose summits were covered with yellow crusts. They were distributed on the scalp, forehead, chin, neck, shoulder, back, and buttocks. The lesions appeared in crops, accompanied by severe pain in the bones and

swelling of all the joints. The bones of the fingers were markedly enlarged. He took 24 doses, and after nine days in the hospital the symptoms disappeared and the lesions healed.

*Case VI.*—S. P., 17 years old, male, had yaws for a month. There were papules covered with a yellowish crust in the right nostril, and there were also scars. The patient entered the hospital on account of pain in the bones and swelling in the joints. He said he had had a similar attack one year ago. He took 14 doses in six days. The treatment caused amelioration of the pain and of the lesions.

*Case VII.*—A. M., male, 40 years old, contracted yaws four years ago. He came for treatment of an old ulcer on the right forearm. It measured about 4 centimeters in diameter. The edges were irregular and elevated, and the base was covered with yellowish purulent material. The patient stayed in the hospital twenty-seven days, during which time he took 80 doses. The ulcer had completely healed when he was discharged from the hospital.

*Case VIII.*—L. S., male, 31 years old, had yaws for one year and four months. The lesions were granulomatous nodules of from 1 to 1.5 centimeters in diameter. They were found on the neck and chest. The face showed numerous pigmented scars. The patient was given 10 drops of a saturated solution of potassium iodide three times daily for nine months. The iodide produced only insignificant improvement of the eruptions on the face. Castellani's mixture was then given, and in five days the lesions completely healed.

*Case IX.*—F. C., female, 40 years old. The patient said she had had an eruption eight years ago. She came for treatment of an ulcer on the right external malleolus. The ulcer was of five months' duration. It was about 4 centimeters in diameter and was covered with fetid whitish material; the border was elevated. The ulcer completely healed after the patient had taken 39 doses in a period of fifteen days.

*Case X.*—F. de G., 40 years old, male, had yaws for one year. The lesion appeared as an abrasion of the skin of the forehead. It later developed into an ulcer. There were four ulcers on the forehead, from 5 to 10 centimeters in diameter, at the time of treatment. The edges were elevated and granular. Castellani's treatment caused complete cicatrization of the ulcers.

*Case XI.*—C. S., female, 16 years old, was ill for four months. At first she had a febrile pain in the joint and itching of the skin over the left knee. The skin then became covered with a yellow crust, which she thought was scabies. Later small papules appeared around the lesion on the knee. These spread in two weeks all over the body and assumed the characteristic appearance of the manifestation of yaws. The patient believed she had contracted the disease from a visitor who had the same disease. She took 47 doses in eighteen days, and the lesions completely disappeared.

*Case XII.*—J. F., 34 years old, was the mother of case XI. She had yaws for three months. The lesions consisted of granulomatous papules on the face and hands and of scaly eruptions on the forearm. The latter were arranged in the form of islands 3 to 4 centimeters in diameter. She was admitted with case XI. After 41 doses, given in sixteen days, she recovered completely.

*Case XIII.*—J. T., 27 years old, married, female, had yaws for three months. The mother yaw was found on the left middle finger. Seven days later granulomatous papules appeared on the face and other parts of the



body. She complained of pain in the bones and joints, but no swelling was found. She stayed in the hospital twenty-three days and took 66 doses. When she was discharged, all the eruptions had completely healed. The last three cases were relatives living in one house. Their infection seems to originate from the visitor mentioned in case XI.

*Case XIV.*—F. O., male, 8 years old. He developed secondary yaws nine months ago. On examination there were found secondary lesions, especially on the penis and about the anus, and an ulcer with granulomatous, irregular border on the left internal malleolus. The diagnosis was tertiary yaws. Thirty doses produced complete cicatrization of the ulcers.

*Case XV.*—E. C., female, 42 years old, had yaws in 1898. At the time of the examination she presented an ulcer on the posterior surface of the right forearm below the elbow. The surface of the ulcer was granular and was covered with fetid, purulent grayish secretion. It measured about 8 centimeters in its longest, and 5 centimeters in its shortest, diameter. It began as an induration of about 0.5 centimeter. It gradually increased in diameter to about 3 centimeters. The diagnosis was tertiary yaws. The ulcer healed without local treatment after the patient had received 30 doses of Castellani's mixture.

*Case XVI.*—I. P., female, 30 years old, had had yaws at the age of 7 years. About three years ago she was hit on the right knee. A nodule appeared on this knee. Later it became an ulcer with deep, irregular borders and fetid yellowish gray secretion. The joint was swollen and painful, incapacitating the patient for walking. The diagnosis was tertiary yaws. She was given 36 doses of Castellani's mixture, and the ulcer completely healed.

The treatment of Castellani undoubtedly exerts a curative influence on the various manifestations of frambœsia. In six or seven days of treatment the granuloma takes on a livid appearance, becomes surrounded by a pinkish halo, and begins to flatten. The crust dries and gradually disappears, leaving a macule that eventually disappears. In deep and extensive ulcers cicatrization takes place gradually and concentrically, the entire process lasting from fifteen to thirty days. Pain in the bones, pain and swelling of the joints, and pruritus are relieved early in the treatment, although sometimes the pain in the bones and joints recurs even after complete healing of the lesions.

The relapses and incomplete recovery observed in some cases are to be ascribed to an incomplete medication, due to the suspension of treatment before the destruction of *Spirochæta pertenuis* was complete. Some of our cases discontinued the treatment after the disappearance of granulomata and ulcers; while others, who were not under our direct supervision, could take only a few doses irregularly. We were not able to supply the patients with the adequate amount of the medicine, because the experiments were carried out in neighboring towns, where we had to distribute the preparation gratis. On the whole, the

patients do not seem to object to this treatment; on the contrary, they willingly submit themselves after they are convinced of its gratifying results.

Our results confirm the conclusion of Castellani that the diverse manifestations of frambœsia heal under the influence of his treatment. The cure of recent infections by this preparation is nearly as marvelous as that by salvarsan and neosalvarsan. We cannot assert as yet whether or not the cure is permanent, since only a limited number of cases remained under our observation for a long time. We believe, however, that the continuation of the treatment after the lesions have healed (from five to ten days' treatment with intermissions of from ten to fifteen days) will insure a permanent cure.

The tendency of the mixture to cause emesis and iodism is not a serious objection to its routine employment, because these untoward effects are slight and may be easily corrected. If emesis, gastralgia, and diarrhœa are troublesome, they can be prevented by merely increasing the sodium bicarbonate or by giving, fifteen minutes before each dose, 4 cubic centimeters of paregoric or 1 centigram of codeine. Other untoward effects, which are apparently due to vasomotor disturbances, such as edema, lachrymation, coryza, congestion of the conjunctiva, etc., are readily overcome by epinephrine. According to Milian, (3, 4) this drug also gives excellent results in iodism and cerebral symptoms associated with the use of arsenobenzol. He strongly advocates the administration of massive doses of the drug, for example, 3 milligrams per orem twice daily, and if this fails, 1 or 2 milligrams injected subcutaneously or intramuscularly.

In the administration of Castellani's mixture it is always important to bear in mind the possible presence of the larval form of hyperthyroidism and also the greater susceptibility of children to iodides, for large doses may give rise to alarming and very unpleasant side effects.

Frambœsia, though not a grave malady quod vitam, has a tendency to relapse, running a highly chronic course extending for years, and gives rise to rheumatoid pains, osseous deformities, extensive ulceration (which may serve as a portal of secondary infection), and deforming cicatrices, which not only are disfiguring but often more or less seriously incapacitate the patients, school children and adults alike. Admitting that it is not a source of high mortality, it is, however, decidedly a cause of high morbidity, entailing material loss to the individual and to the community.

Framboesia is one of the common diseases in the Philippine Islands and is widely distributed. It is impossible to estimate the extent of infection, but it is undoubtedly of considerable importance. The establishment of ambulatory or provisional hospitals in the larger infested districts for the adequate administration of salvarsan would be the ideal method of suppressing this disease. This being impossible under present conditions, the general use of Castellani's treatment, under suitable administration, offers a practicable means of combating the disease.

We desire to express our gratitude to Doctors Calderon, Lantín, and Reyes and to Messrs. Hallare and Fernandez for cooperation that made this work possible.

#### BIBLIOGRAPHY

1. CASTELLANI, A. *Journ. Trop. Med. & Hyg.* (1915), 18, 61.
2. IDEM. *Brit. Med. Journ.* (1916), 552.
3. MILIAN, G. *Soc. Dermat.* (1913).
4. IDEM. *Paris Medicale* (1917), 343.
5. SPAAR, E. C. *Journ. Trop. Med. & Hyg.* (1915), 18, 170.





## ILLUSTRATIONS

### PLATE I. YAWS

- FIG. 1. Case VIII, before treatment.  
2. Case VIII, five days after treatment.  
3. Case XIII, before treatment.  
4. Case XIII, after 41 doses given in sixteen days.

### PLATE II. YAWS

- FIG. 1. Case XV, before treatment.  
2. Case XV, after 30 doses of the mixture.  
3. Case XVI, before treatment.  
4. Case XVI, after 36 doses of the mixture.

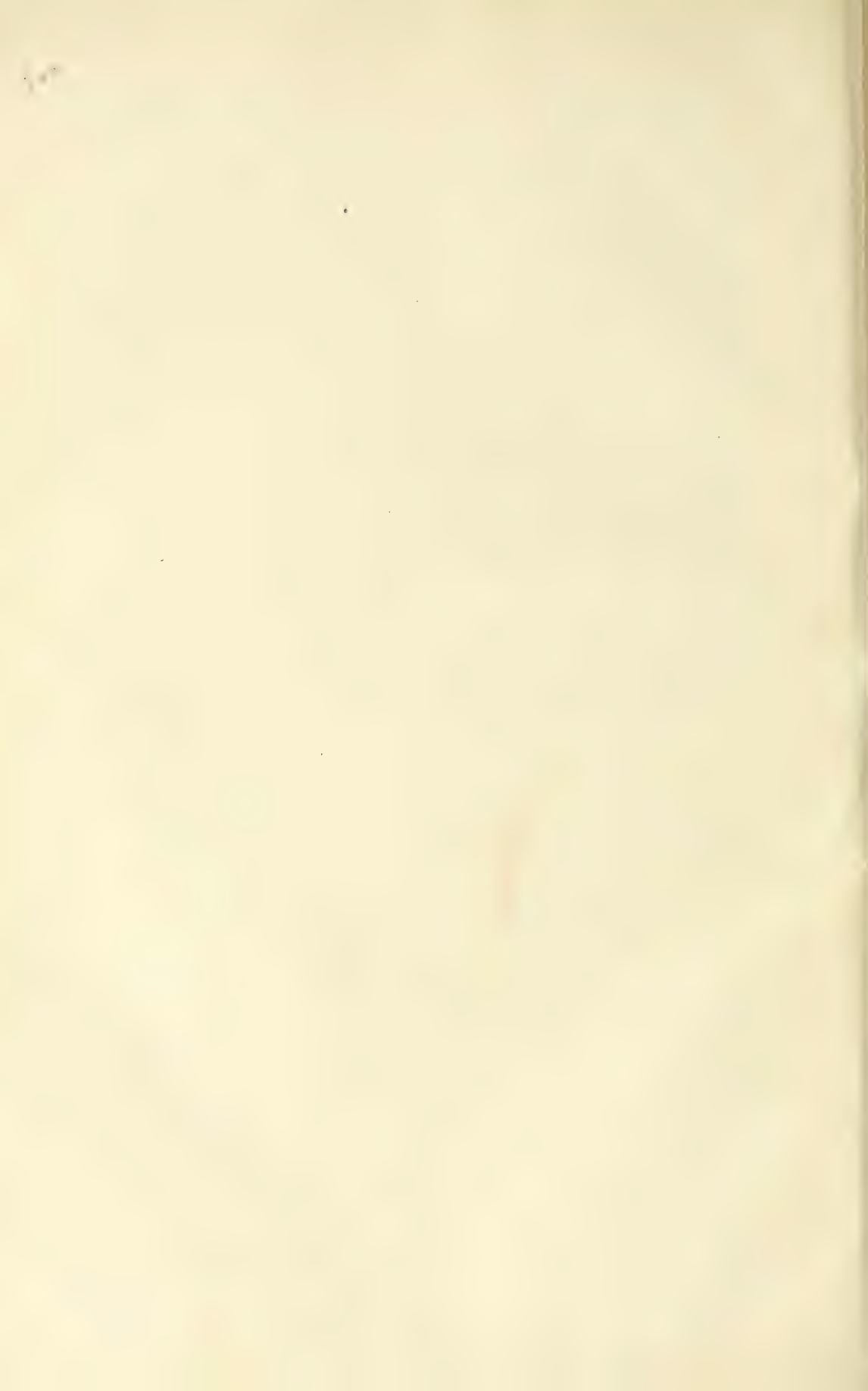






Fig. 1. Case VIII, before treatment.



Fig. 2. Case VIII, five days after treatment.



Fig. 3. Case XIII, before treatment.



Fig. 4. Case XIII, after 41 doses given in sixteen days.

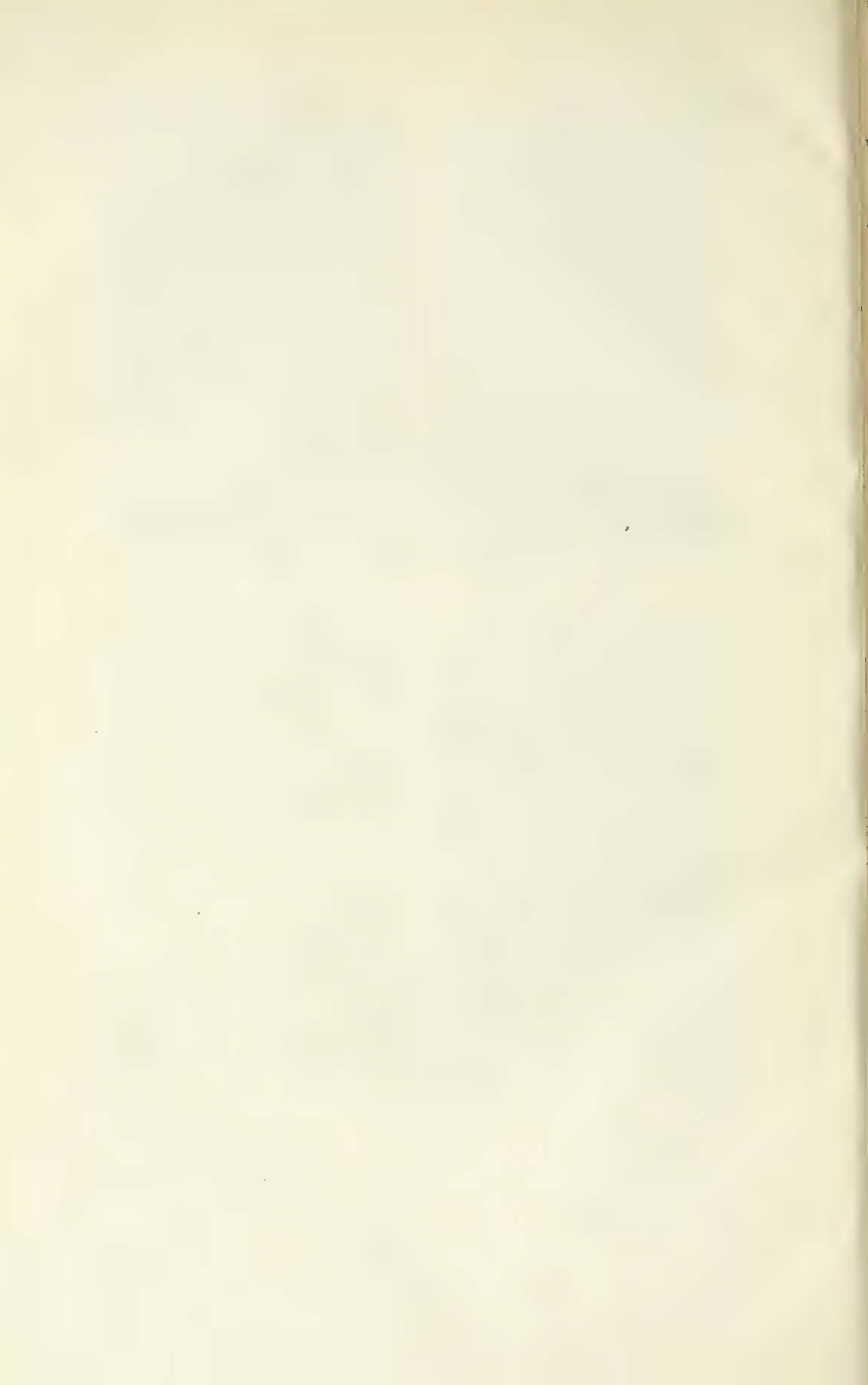




Fig. 1. Case XV, before treatment.

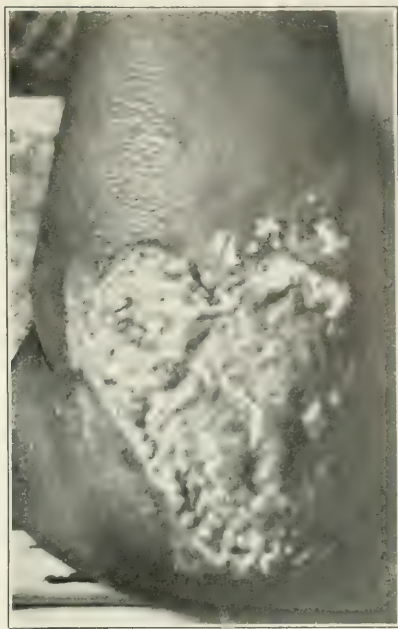


Fig. 2. Case XV, after 30 doses of the mixture.

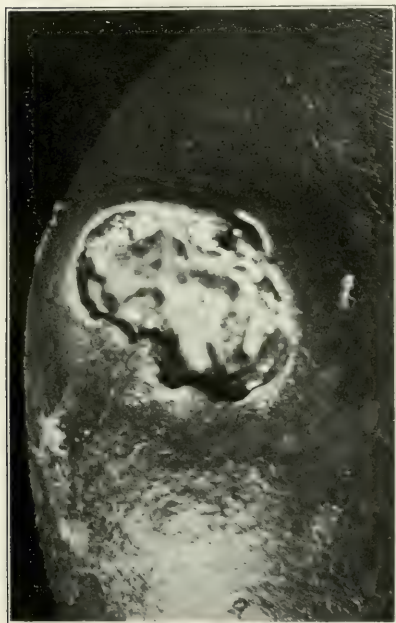
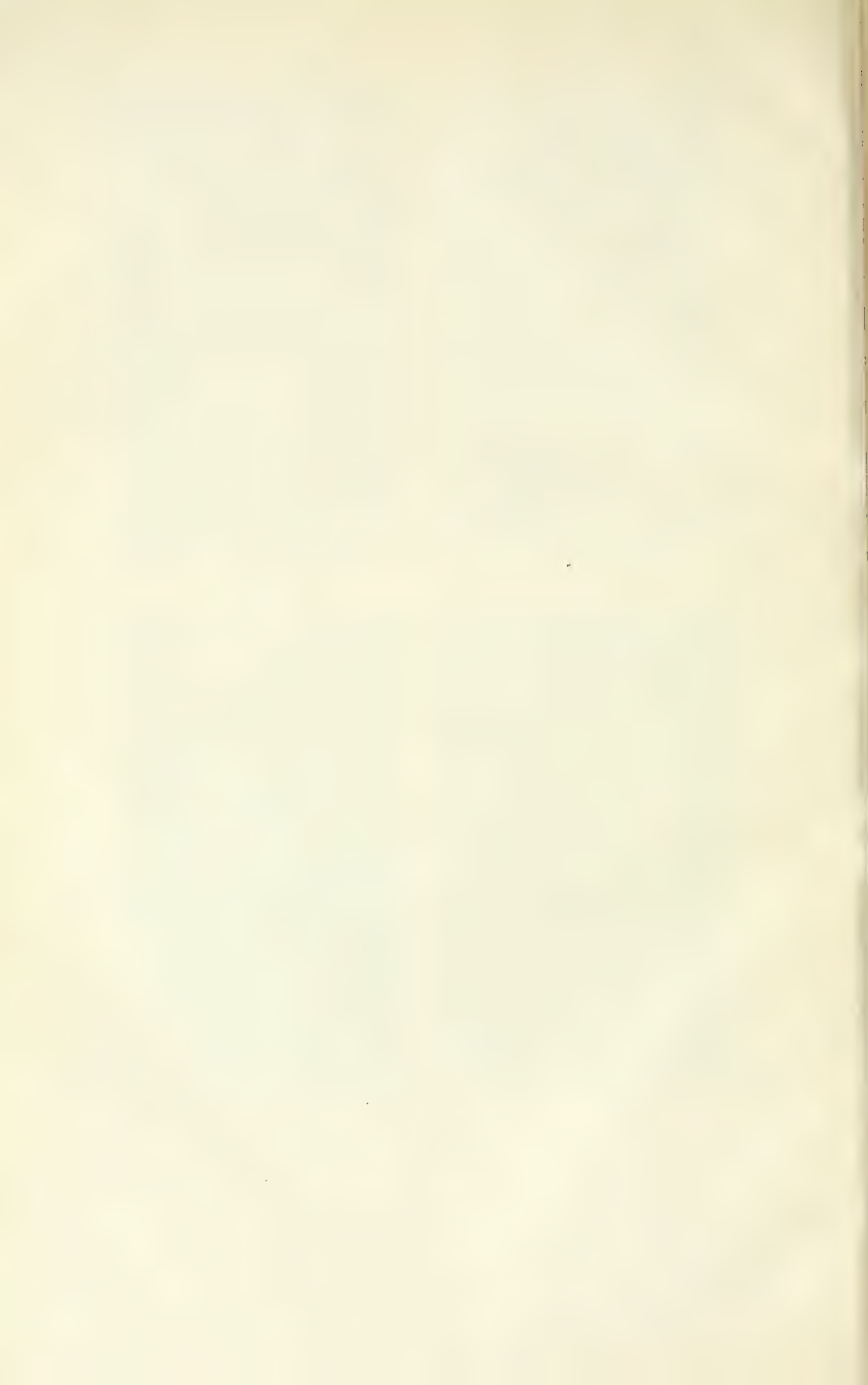


Fig. 3. Case XVI, before treatment.



Fig. 4. Case XVI, after 36 doses of the mixture.





# RECONSTRUCTION OF TWO SETS OF DUCK TWINS, WITH SPECIAL REFERENCE TO THE EARLY EMBRYONIC DEVELOPMENT OF THE VASCULAR SYSTEM<sup>1</sup>

By EDWARD S. RUTH

(From the Department of Anatomy, University of the Philippines)

## TWO PLATES

The early stages of a large series of chick and duck twin embryos have been studied especially by Kaestner,<sup>(2)</sup> and a few have been recorded by Burckhart,<sup>(1)</sup> Reichert,<sup>(4)</sup> and Mitchell.<sup>(3)</sup> However, up to the present time only a few of the embryonic twins have been reconstructed. Kaestner apparently was the pioneer in making reconstructions of young twin embryos. Those figured by him are considerably older than the duck twins under discussion in this paper. Much may be elucidated regarding the early formation and intimate relation of embryonic twins by careful detailed study of the serial sections and especially by making a reconstruction. In a previous communication<sup>(5)</sup> I described four sets of duck twins. Set III of that paper has been reconstructed and is figured in this contribution; set V was found subsequent to the issue of the previous publication. These two sets of duck twins have been reconstructed according to the Borne method. I wish to express my appreciation and thanks to Dr. Ricardo Molina for the reconstruction of duck twins set V.

## DUCK TWINS SET V

The duck twins are in the 9-somite stage of development. The head and thoracic regions lie parallel to each other, while the caudal extremities diverge, forming an angle of about 55°. Surrounding the embryos is the area pellucida, which is transparent. It is more marked on the ventral side of the head in the region of the proamnion.

*Ventral side.*—The heads are abnormally large and somewhat club-shaped. In the region of the fovea cardiaca the membranes have an increased opacity, extending laterad to the

<sup>1</sup> Received for publication October 13, 1917.

embryos and pointing caudad. The former is apparently the position of the omphalomesaraic, and the latter is apparently the position of the median vein. The posterior openings of the foregut are separated from each other by a rounded, narrow partition. On cross section a tubular vessel occupies this space in the partition, separating the posterior ends of the foregut. At the extreme tip of the caudal extremity, remains of the primitive streak are still present.

*Dorsal side.*—The heads project slightly dorsad to the pro-amnion. They are distinctly separated from each other for a short distance where the ectoderm becomes continuous from one head to the other. On the median side of the heads there is a small cleft where the medullary groove has failed to close entirely. This marks the position of the anterior neuropore. The medullary groove has closed over in the cephalic region, caudad to the anterior neuropore in both embryos; however, it again becomes open and remains so throughout its entire extent. The caudal portion of the medullary groove in the right embryo is slightly more expanded than in the left. The somites are regularly developed. The medial ones are somewhat larger than those on the lateral sides of the embryos.

*Reconstruction of duck twins set V.*—This set of twins is somewhat younger than set III. The foreguts are symmetrically developed, the openings of which are at first common to both components, but they are soon divided and separated from each other by a fold of mesoderm. The tubular hearts are indistinctly developed and appear to be double at the middle and caudal third. The omphalomesaraic veins join the tubular hearts, the left vein having a position much more cephalad than the one on the right side. Vaso formative cells link the one tubular heart with the other. On the dorsal side several median veins can be seen lying on the medial anterior wall between the two pharynges. The venous confluence can be made out rather indefinitely; it is not so distinct as in set III. As the single tubes approach the cephalic end of the pharynx, the one on the left component turns abruptly laterally and downward to gain the dorsal side of the pharynx, where it becomes the dorsal aorta. The tubular heart in the right component passes obliquely and laterally over the most cephalic portion of the pharynx to gain the posterior side. Both dorsal aortæ pass caudad along the lateral and dorsal side of the pharynx and are lost in the area pellucida after leaving the embryos. On the dorsal side of the pharynx some distance above the opening of the foregut a median dorsal vein also makes

its appearance (Plate II). This, like the other, is also lost in the interembryonic tissue at a distance slightly more caudad.

#### DUCK TWINS SET III

Reconstruction of duck twins set III shows the foreguts and vascular system symmetrically developed in every detail (Plate II). In the region of the thorax, abdomen, and caudal extremity the ectoderm and entoderm are continued from one embryo to the other. The dorsal aortæ are all distinctly and uniformly developed, though their continuation into the vitalline arteries cannot be traced. In the region of the fovea cardiaca the venous blood vessels form a median confluence by the union of the two omphalomesaraic veins with the median vein, which approaches the embryos from a caudal interembryonic direction. The large median vein is formed from a series of smaller veins beginning symmetrically from eight smaller veins. Extending cephalad to the venous confluence are two tubular projections. The tubular hearts are symmetrically developed. Both hearts, as they approach the cephalic portion of the foreguts, divide into two ventral aortæ, which loop around the lateral sides of the pharynx to form the dorsal aortæ. The dorsal aortæ terminate rather abruptly in the region of the venous confluence on the posterior side of the foregut. No vitelline arteries appear to be present. The medullary tubes and grooves are entirely separate and distinct from each other throughout the entire extent of the embryos; both are symmetrically developed. On the ventral side of each medullary tube is a notochord. The foreguts are uniform in every detail. Each component in this set of twins is separately developed, with the exception of the venous confluence. Here the two embryos appear to have a vascular chamber in common (Plate II). All other structures at this stage of development are duplicated.

#### DISCUSSION

The two sets of twins have a number of developmental parts in common. However, in set V the medial dorsal arteries are only partially developed, while the lateral have already joined with the ventral aortæ. In the older twins the blood vessels have apparently assumed a fixed position in relation to the embryo, while in the younger twins they are in a migrating stage, that is, in the younger twins the heart anlage has not yet become tubular in several areas. In both twins there is present a venous confluence at the proximal end of the tubular hearts formed by two omphalomesaraic veins and a median vein.



Kaestner shows a similar confluence in a set of his chick twins, but does not show a median vein. Furthermore he considers the confluence in the process of developing into a single heart and concludes that in all probability if growth had continued there would have developed a single heart. The confluence of the veins does not correspond to the position of the heart in embryos of this stage of development, but from the single tubular heart that lies more cephalad. If any conclusion can be drawn, one heart should develop from each tubular heart, as this corresponds to the region where it normally develops.

## REFERENCES

1. BURCKHART, R. Doppelanlage des Primitivstreifens bei einem Hühnerei. *Arch. f. Anat. u. Physiol.—Anat. Abth.* (1888), 431.
2. KAESTNER. Doppelbildung an Vogelkeimscheiben. *Ibid.* (1902), 117.
3. MITCHELL. On a double chick embryo. *Journ. Anat. & Physiol.* (1890–91), 25, 316.
4. REICHERT. Anatomische Beschreibung dreier, sehr frühzeitiger Doppel-Embryonen von Vögeln,—zur Erläuterung der Entstehung von Doppel-Missgeburten. *Arch. f. Anat., Physiol. u. wiss. Med.* (1864), 744.
5. RUTH, E. S. On the development of twins and other polyembryos, with especial reference to four sets of duck twins. *Phil. Journ. Sci., Sec. B* (1916), 11, 109.



## ILLUSTRATIONS

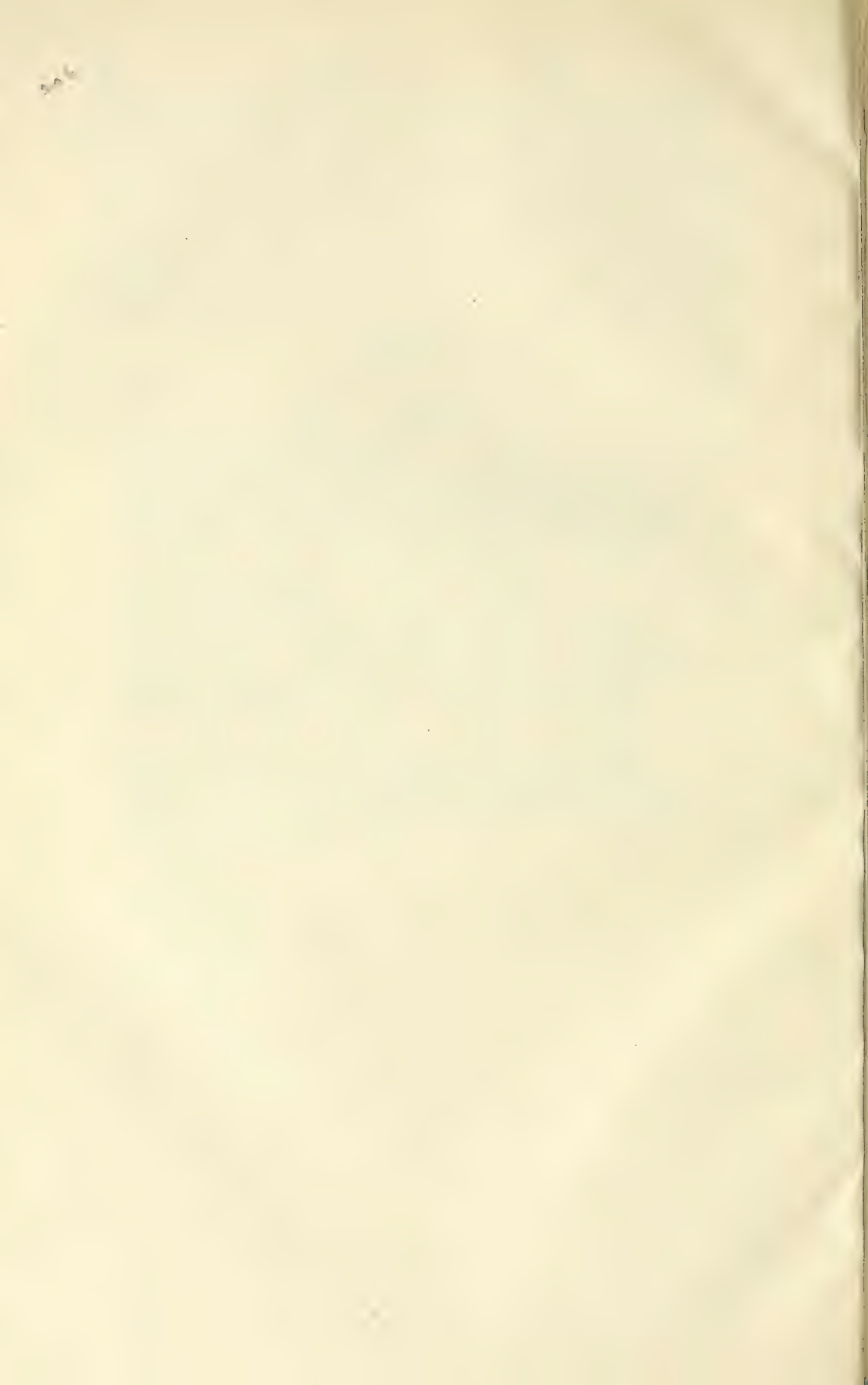
[Drawings by J. Castro.]

### PLATE I

- FIG. 1. Ventral view of duck twins V. *pa*, proamnion; *fc*, fovea cardiaca; *ls*, lateral somite; *cd'*, *cd*, chorda dorsalis; *ps*, primitive streak.
2. Dorsal view of duck twins V. *pa*, proamnion; *an*, anterior neuropore; *mg'*, *mg*, medullary groove; *ls*, lateral somite; *mv*, median veins; *ap*, area pellucida.

### PLATE II

- FIG. 1. Dorsal view of pharynx and aortæ from duck twins III. *ph'*, *ph*, pharynx; *da'*, *da*, dorsal aortæ; *omv'*, *omv*, omphalomesaraic vein; *mv*, median vein; *veh*, venous end of heart.
2. Ventral view of pharynx, tubular hearts, and aortæ from duck twins III. *ph'*, *ph*, pharynx; *th*, tubular heart; *ve*, ventral end of heart; *va*, ventral aorta; *omv*, omphalomesaraic veins; *mv*, median vein.
3. Ventral view of pharynx and aortæ, duck twins V. *omv'*, *omv*, omphalomesaraic vein; *ph*, pharynx; *th'*, *th*, tubular heart; *of*, opening of foregut; *mv*, median vein.
4. Dorsal view of pharynx and aortæ, duck twins V. *lda'*, *lda*, lateral dorsal aortæ; *ph*, pharynx; *mda*, median dorsal aortæ; *mv*, median vein.



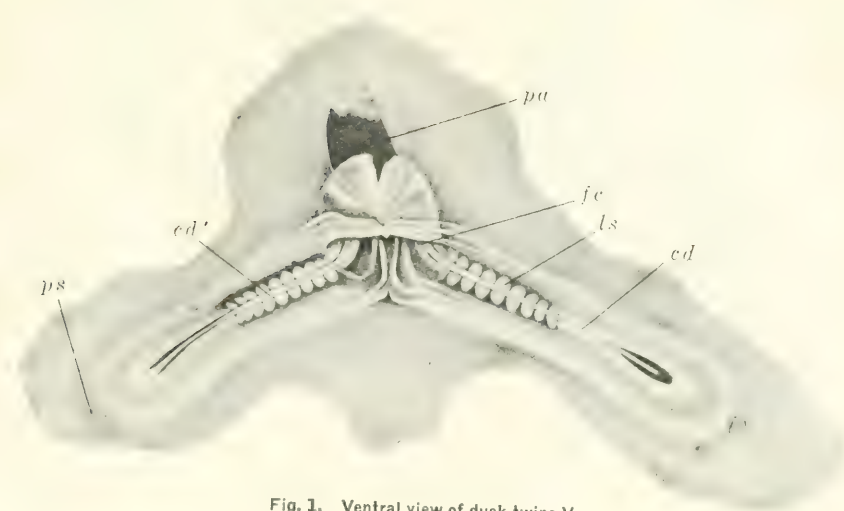


Fig. 1. Ventral view of duck twins V.

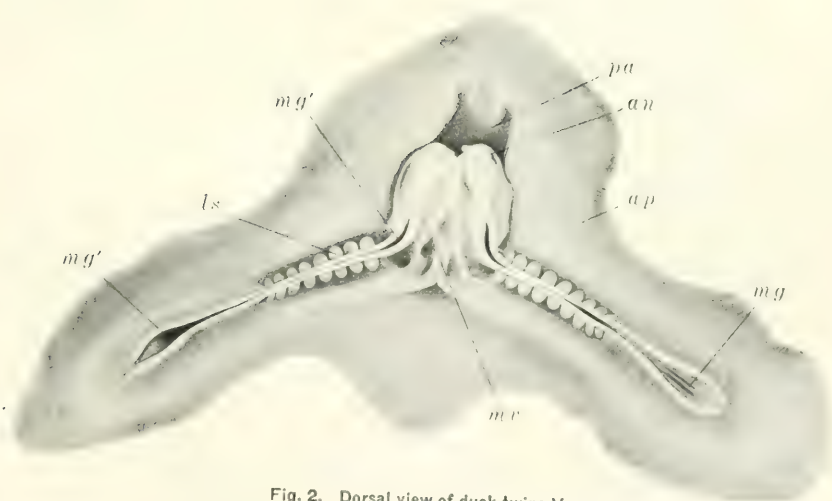
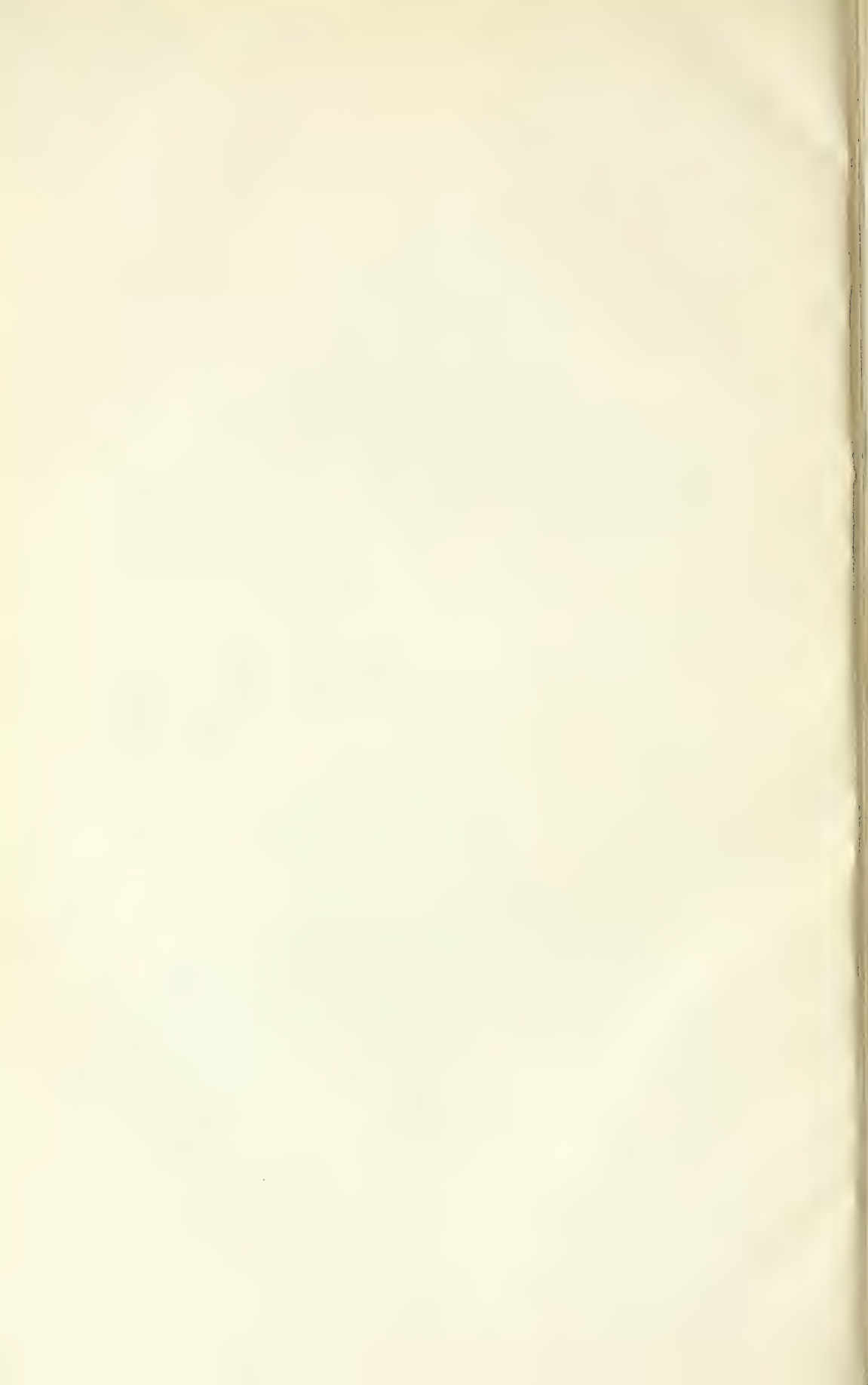


Fig. 2. Dorsal view of duck twins V.

PLATE I.





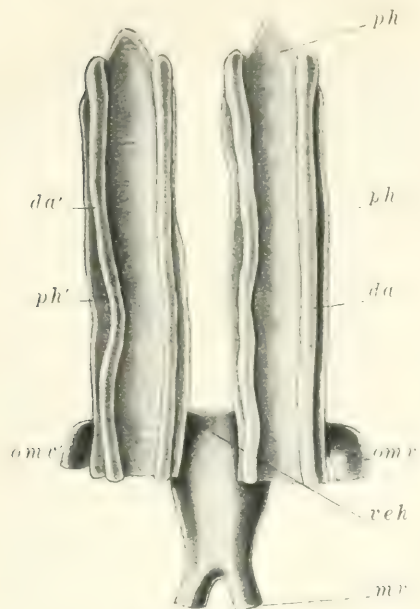


Fig. 1. Dorsal view of pharynx and aortæ.  
Duck twins III.

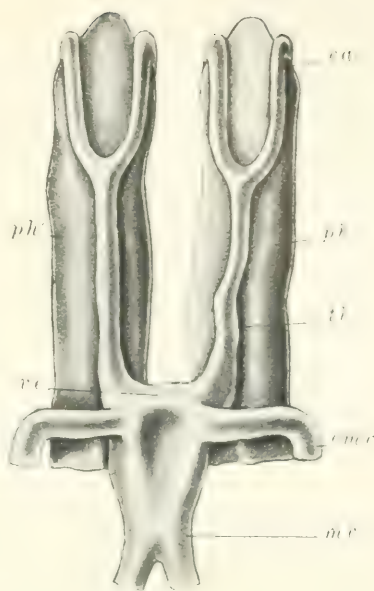


Fig. 2. Ventral view of pharynx, tubular heart,  
and aortæ. Duck twins III.

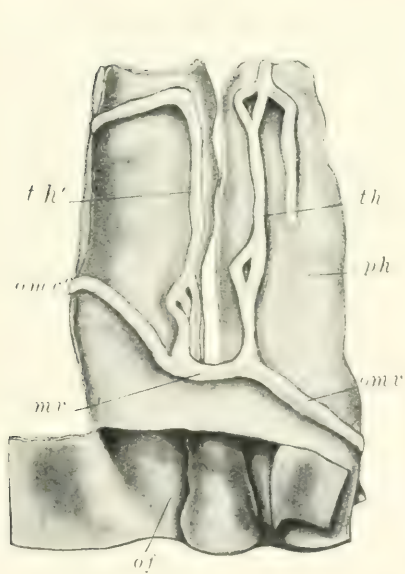


Fig. 3. Ventral view of pharynx and aortæ.  
Duck twins V.

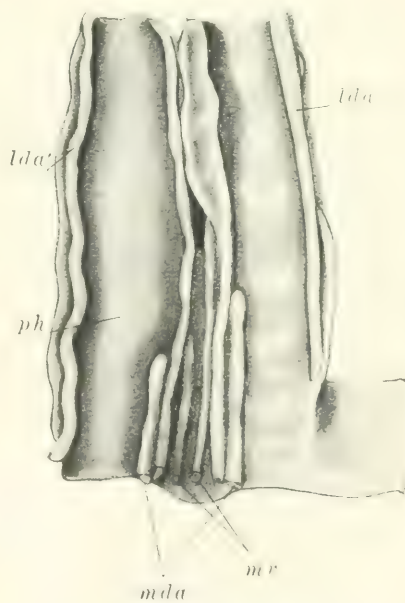
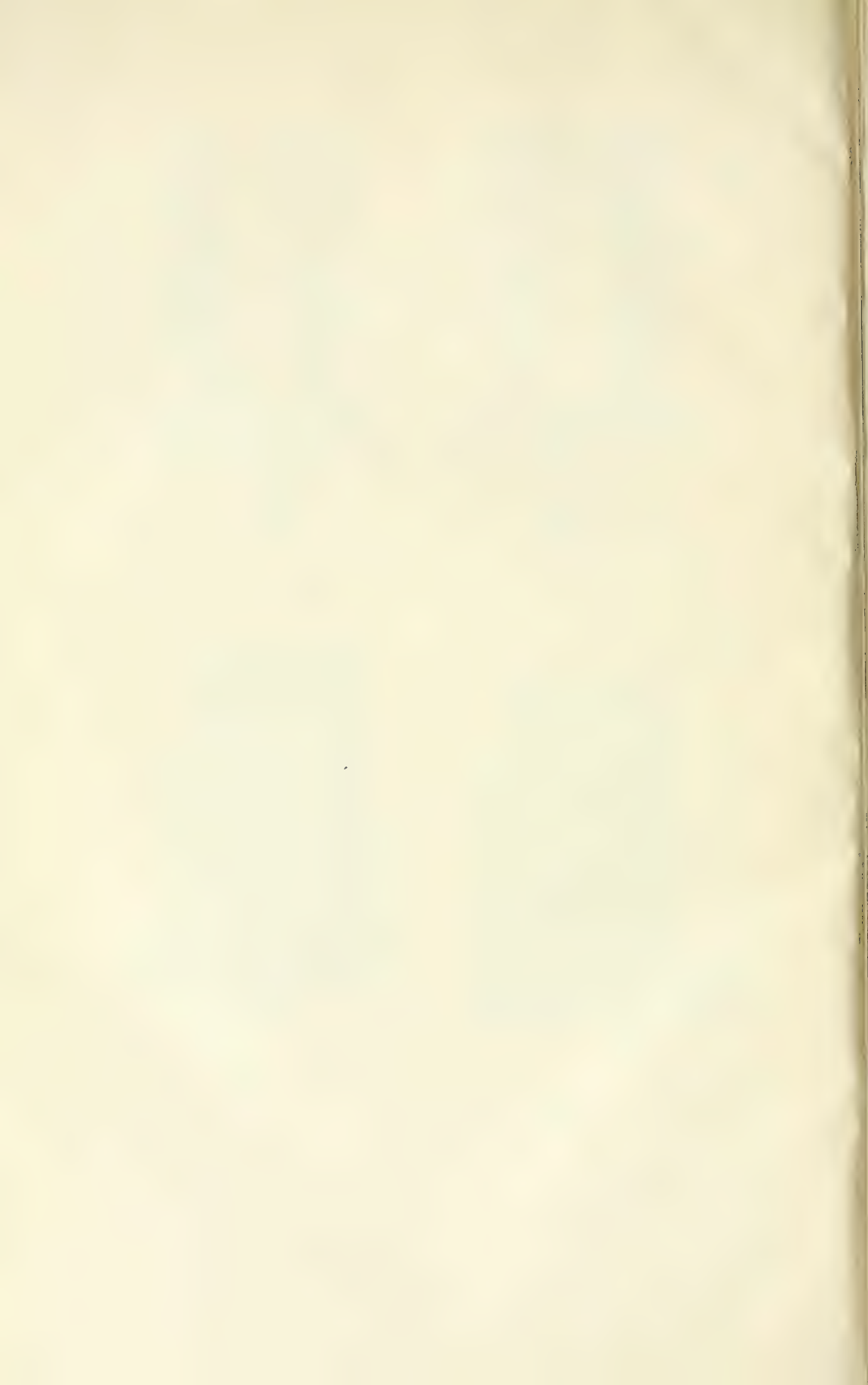


Fig. 4. Dorsal view of pharynx and aortæ.  
Duck twins V.



## REVIEWS

The Prescription | therapeutically, pharmaceutically, grammatically and |  
historically considered | by | Otto A. Wall, Ph. G., M. D. | [7 lines] |  
fourth and revised edition | St. Louis | C. V. Mosby Company | 1917 |  
Cloth, pp. 1-274. Price, \$2.50.

An extremely interesting and readable book, but not one commending itself as a textbook. The desire of the publishers to present a typographically perfect book has apparently been well carried out. The use of the term "yelk" for yolk of egg on page 229 is very questionable.

J. A. J.

Volume I Johns Hopkins Number | Number I | The | Medical Clinics | of |  
North America | July, 1917 | Published bi-monthly by | W. B. Saunders Company | Philadelphia and London | [6 lines]. Pp. 1-193.  
Price, per year: Paper, \$10 (foreign, £2 2s); cloth, \$14.

The first number of The Medical Clinics of North America gives one a favorable impression. The feature in this publication is the question and answer portion by which means the lecturer's ideas on diagnosis and treatment are forcibly brought before the reader.

J. A. J.





## PROCEEDINGS OF THE MANILA MEDICAL SOCIETY

REGULAR MONTHLY AND ANNUAL MEETING, JANUARY 7, 1918

### MINUTES OF THE MANILA MEDICAL SOCIETY

The regular monthly and annual meeting of the Manila Medical Society was held in the College of Medicine and Surgery on Monday evening, January 7, 1918, at 8.30, with President Ruth presiding. There were 18 members and 2 visitors present.

The minutes of the last meeting were read and approved as read.

The applications for active membership of Drs. A. Francis Coutant, Emiliano M. Panis, and Isidoro de Santos were presented with the recommendation of the council. It was moved and seconded that the secretary be instructed to cast a unanimous ballot in favor of the above applicants. The motion was carried.

The program for the evening was then carried out as follows:

1. Glioma Retinæ, by Dr. Herminio E. Velarde.
2. Poisonous Snakes of the Philippines, by Mr. E. H. Taylor.
3. A Report of Five Cases of Leukemias Clinically Diagnosed as Splenomegaly, by Dr. José S. Hilario. (This paper was read by title.)

The annual report of the secretary-treasurer for 1917 was read and approved as read.

The president next appointed Drs. B. C. Crowell, R. B. Gibson, and José Albert as a committee on nominations for the officers of the society for the ensuing year. The meeting was suspended for a few minutes pending the return of the nominating committee.

The nominating committee then returned and recommended the following:

For President:	Dr. Otto Schöbl.
For Vice-President:	Dr. H. G. Maul.
For Secretary-Treasurer:	Dr. Daniel de la Paz.
For Councilor for three years to succeed Dr. J. E. Reed, Jr.:	Dr. E. S. Ruth.
For Councilor for five years:	Dr. A. P. Goff.

There being no objection to the nominations, it was moved, seconded, and carried that the election of the above-named officers as recommended by the committee on nomination be approved.

The newly elected officers were then informally installed, and in the absence of President Schöbl, Vice-president Maul presided.

On motion duly made and seconded, Doctor Gibson was unanimously elected editor of the proceedings, and a vote of thanks by the society was extended him for his excellent and proficient work in the past year.

A vote of thanks was also extended to the outgoing officers.

There being no further business, it was announced that there would be no meeting in February, because of the joint meeting of the Philippine Islands Medical Association and the IV Asamblea Regional de Médicos y Farmacéuticos de Filipinas, and the meeting adjourned.

H. G. MAUL,  
*Secretary-Treasurer.*

#### SCIENTIFIC PROGRAM

##### SIX CASES OF GLIOMA RETINÆ

By DR. HERMINIO E. VELARDE

Case histories, illustrated with photographs and skiagrams, of six cases of glioma retinæ were presented. All six cases were females, the ages being from 1½ to 4 years; the condition in one case was observed shortly after birth. Cases reported in the literature have occurred in children from 10 weeks to 13 years of age. Other members of the families were not affected. In two out of the six cases the condition was bilateral; the usual figure is 25 per cent of bilateral cases. Metastatic involvement of the liver was observed in two cases, of the scalp in two cases, and also of the cervical glands, ribs, shoulders, thighs, pleura, and dura mater. The prognosis of the disease is serious and almost certainly fatal, as exemplified by the present series, if early enucleation is not done.

##### POISONOUS SNAKES OF THE PHILIPPINES

By MR. E. H. TAYLOR

Of the 25 deadly poisonous snakes known in the Islands, 18 species were shown in the demonstration. Of the Crotalinae, or pit vipers, the following were examined: *Trimeresurus gramineus*, from Luzon; *T. flavomaculatus*, from Luzon; *T. schultzei*, from Palawan; *T. wagleri* (and two varieties), from Mindoro and Palawan; *T. haliens*, a rare species from Polillo.

A new yellow viper (recently discovered on Batan Island, Batanes Islands), as yet undescribed, was shown.

The poison apparatus of this group was discussed, and the large fangs, which attain a greater size in this group than in any other, were observed and discussed.

Of the Elapidæ, which are the most deadly of reptiles, good specimens of the following were shown: *Naja hannah*, the king cobra, or hamadryad; *N. naja caeca*, from Luzon; *N. n. miolepis*, from Palawan; and *N. n. samarensis*, from Mindanao.

The last three are the Philippine representatives of *Naja naja tripudians*, the famous spectacled cobra of southern Asia. These species are known in Luzon as *alopong*, or *carasaen*, the latter name being used in the north in the Ilocos provinces and Pangasinan. They may be readily recognized, when alive, by the habit of spreading the neck, and in the three subspecies they may be recognized by the loud hissing noise made. They are able to throw the poison to a distance of at least two meters.

Of the related genera, specimens of *Hemibungaris* and *Doliophis* were displayed. Special attention was called to the unique poison glands found in the latter genus. Instead of being situated in the head, they form long club-shaped glands, which are contained in the body cavity; these attain a length in adults of at least 20 centimeters.

Specimens of the aquatic genera *Disteira*, *Lapemis*, and *Laticauda* were shown. These are the sea snakes; they occur in large numbers in Manila Bay.

The Opisthoglypha (poisonous snakes not regarded as deadly) were represented by several species of *Boiga* and by the color varieties of *Dryophis prasinus*, the widely famed *dahon palay*.

A few harmless forms were discussed that approximate the poisonous forms in color, markings, and habits, and so are very frequently confused with them.

It was requested that more careful observations be made and records be kept by provincial doctors of cases of snake bites, that effort be made for a more careful recording of statistics of death from snake bites by health authorities, and that antivenin serums be manufactured in the Islands.

R. B. GIBSON,  
*Editor of the Proceedings,*  
*Manila Medical Society.*





## PROCEEDINGS OF THE MANILA MEDICAL SOCIETY

REGULAR MONTHLY MEETING, MARCH 4, 1918

### MINUTES OF THE MANILA MEDICAL SOCIETY

The regular meeting of the Manila Medical Society was held at the College of Medicine and Surgery, March 4, 1918, at 8.30 in the evening.

The following members were present:

Dr. B. C. Crowell.	Professor F. G. Haughwout.
Dr. E. S. Ruth.	Dr. H. W. Wade.
Dr. F. W. Vincent.	Dr. J. S. Hilario.
Dr. R. B. Gibson.	Dr. F. Garcia.
Dr. B. Samson.	Dr. D. de la Paz.

The secretary announced the absence of the president and vice-president and with the consent of the society appointed Dr. B. C. Crowell to officiate as temporary chairman.

The minutes of the previous meeting were read and approved.

A letter from the President-elect, Dr. Otto Schöbl, declining the acceptance of the presidency of the society, was read and accepted with regret.

Doctor Crowell appointed Doctors Ruth, Gibson, and Samson to constitute a committee on the nomination for the president.

The society took a short recess.

At the second session the committee announced that Dr. F. W. Vincent was unanimously nominated. There being no other nominations, the secretary was instructed to cast a unanimous ballot in favor of the nomination.

With President Vincent in the chair, the reading of the papers was then proceeded with.

The first paper, entitled Studies of Cerebrospinal Fluid in Acute Anterior Poliomyelitis, by John A. Kolmer and co-workers, abstracted by Dr. John A. Johnston, was read by Doctor Gibson in the absence of Doctor Johnston.

Doctor Wade presented his paper, entitled Cryptoplasmic Infection, which was discussed by Professor Haughwout and Doctor Crowell, Doctor Wade closing the discussion.

The last paper, entitled The Prophylaxis of Malaria, was read

by its author, Doctor Samson. It was discussed by Professor Haughwout.

The society adjourned at 9.55 in the evening.

D. DE LA PAZ,  
*Secretary-Treasurer.*

#### SCIENTIFIC PROGRAM

### STUDIES OF THE CEREBROSPINAL FLUID IN ACUTE ANTERIOR POLIOMYELITIS<sup>1</sup>

By JOHN A. KOLMER and co-workers

Abstracted by DR. JOHN A. JOHNSTON

The results summarized in this paper are based upon the examinations made in 868 specimens of cerebrospinal fluid obtained during the epidemic in Philadelphia during 1916.

A summary follows:

1. The majority of the fluids were water clear or but faintly opalescent when viewed against a black background.

2. Seventy-seven per cent of the fluids showed an increase of total cells.

3. In over 96 per cent of the fluids from cases after the onset of paralysis the small lymphocyte variety of cells predominated. Polymorphonuclear cells predominated in less than 1 per cent of the fluids, and in over 88 per cent they constituted less than 25 per cent of the cells present.

4. An increase of protein was found in from 32 to 42 per cent of fluids. With the Noguchi test the fluid of 1 of 6 cases in the preparalytic stage yielded a positive reaction. The percentage of positives then became gradually higher to the third week after the onset of paralysis, when a rapid decrease in protein became apparent.

5. During the acute stages of poliomyelitis the fluids of 40 to 50 per cent of cases yielded a colloidal gold reaction of the luetic and meningitic zone types.

6. The potassium permanganate reduction test yielded positive reactions of indices over 2.3 with the spinal fluids of 41 per cent of cases examined from the second to twenty-first days after the onset of paralysis.

Every fluid was found to contain sufficient dextrose to reduce Fehling's solution in some degree.

8. Increased permeability of the meninges was indicated by the presence of natural antishoop hemolysis in the fluids of 66 per cent of cases in the acute stages and both the hemolysis and

<sup>1</sup> *Am. Journ. Med. Sci.* (1917), 144, 720-733.

a hemolytic complement in 30 per cent. Both of these substances were absent in the fluids of controls.

9. Substances inhibiting saponin hemolysis were not found in the fluids of cases, two to twenty-one days after the onset of paralysis.

10. A definite and absolute diagnostic criterion or laboratory test has not been discovered. A clear or slightly opalescent fluid flowing under increased pressure, sterile as examined by smear and culture when collected aseptically, poor in fibrin, reducing Fehling's solution, and containing an increased number of cells chiefly of the mononuclear variety are the most constant findings. An increase of protein and a high potassium permanganate reduction index strengthen the diagnosis, while a colloidal gold reaction of the luetic and meningitic zone types and the presence of natural antisheep hemolysis are helpful diagnostic data.

#### CRYPTOPLASMIC INFECTION

By DR. H. W. WADE

The findings in cultures of material from a group of ulcers of undetermined causation seen in Hagonoy, Rizal, were described. Spirochætal, "blastomycotic," etc., etiology was negatived by absence of the characteristic microscopic findings, and yaws and syphilis could, it was thought, be eliminated.

In certain of the cultures of tissue fragments on special media the tissue cells, whether in spite of or because of overgrowth by bacteria contaminating the lesions, underwent apparently additive changes that sometimes produced deeply staining bodies not ordinarily seen in such material. These were referred to as "basic" bodies. In one culture from each of two cases, after considerable time, a *Cryptococcus* was found.

This fungus, classifiable as a *Cryptococcus*, was of an unusual type. It was not in the least saprophytic, did not even produce visible colonies, and did not multiply in subcultures. Therefore it could not be isolated.

The source of the fungus was not apparent. It was suggested that, in view of its late appearance in the cultures and its very unusual lack of cultivability, it might have arisen from the basic bodies. In this case it may have been concerned in the etiology of the lesions in an unrecognizable form.

These observations constitute a contribution toward the establishment of a hypothetical "cryptoplasmic" mode of infection. According to this hypothesis, which refers primarily to certain fungus infections, an invading organism may, whether occa-

sionally or regularly, wholly or in part, lose its morphologic, though retaining its biologic, identity and may exist and act in the form of a protoplasmic mass that, if it is not actually amorphous, is at least of such low morphological differentiation as not to be demonstrable. This substance is referred to as a "cryptoplasm." Other observations that have contributed to the formulation of this hypothesis are to be reported.

#### PROPHYLAXIS OF MALARIA

By DR. BERNARDO SAMSON

Doctor Samson outlined his methods of treating malaria both from the curative and the prophylactic viewpoints. He stated that one of the principal difficulties in connection with the prosecution of antimalarial campaigns in the provinces lay in the difficulty of establishing an understanding between the people and the health officers. The distribution of pamphlets printed in English, in Spanish, and in the vernacular he regarded as of doubtful utility. Better work, he thought, could be done by carefully arranged lectures, illustrated by lantern slides, given by the Bureau of Health officials or local physicians. Instruction in hygiene in the public schools, he believed, should include more information on mosquitoes and the symptoms and consequences of malaria. Doctor Samson said he thought much good would come by enlisting the coöperation of hacenderos employing large numbers of laborers on their plantations. He advocated the free distribution of quinine.—F. G. H.

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THE TISSUE-INVASIVE POWERS OF THE FLAGELLATED AND  
CILATED PROTOZOA WITH ESPECIAL REFERENCE TO  
TRICHOMONAS INTESTINALIS. A CRITICAL REVIEW\*

By FRANK G. HAUGHWOUT<sup>1</sup>

(From the Department of Medical Zoölogy, College of Medicine and Surgery,  
University of the Philippines)

### ONE TEXT FIGURE

The recent work of Hadley, (20) in which he has demonstrated that under certain conditions, so far unascertained, *Trichomonas* may become a destructive cytozoic and histozoic parasite, has again brought up the question of the tissue-invasive powers and pathogenicity of the flagellated and ciliated protozoan parasites found in the intestine of man. The question is of such prime importance in the practice of medicine, particularly in the tropics, that it seems opportune at this time to review some of the literature on these very important subjects. It must be confessed at the beginning that it seems impossible to draw any very definite conclusions; but force is added to the already very prevalent impression that the flagellated intestinal protozoa should be viewed with suspicion and regarded as pathogenic until the contrary is proved beyond dispute.

From the viewpoint of human pathology the matter can be scarcely discussed at this time, for with the exception of *Balantidium* infections of the human intestinal tract there are no observations upon which to work. The whole problem from the clinical, pathological, and experimental viewpoints presents ex-

\* Received for publication March 15, 1918.

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ceptional difficulties, both to the pathologist and to the proto-zöologist.

A review of the situation brings out some points in parasitology of great interest from both the theoretical and practical viewpoints and raises some questions that promise to form the basis for an interesting series of studies. Among other questions raised is that that bears on the effects produced upon a parasite by the internal reactions of the host, as expressed through the tissues and body fluids. In this connection I mean reactions of the host that tend to favor the parasite as distinguished from the familiar reactions that work to the disadvantage of the parasite. In the past the attention of parasitologists and physicians has been focused largely upon the effects of the parasite upon its host—a most natural point of view. But in reviewing the subject, especially in the light of recent evidence, it is hard to escape the impression that the host, on occasion, may transform an apparently harmless parasite into one that is pathogenic or even lethal to its host. This is entirely apart from conditions of lowered vitality and resistance, which proverbially favor the development of infections of all kinds. It would seem that the term “harmless commensal” has been very much overworked. The case of the intestinal nematode *Ascaris lumbricoides* is very much in point, and apparently the day is not far distant when the terms “harmless commensal” and “symbiont” will be used in the literature with much greater caution than has been shown in the past. It has long been my belief that time would show that all animal parasites that have been regarded as commensals and symbionts in the alimentary tract, if not actually giving rise to lesions, would yet be shown to affect unfavorably the physiological balance of the host in some way.

Recently this has been strikingly indicated by Gibson,(17) who has noted the apparent restraining influence of *Ascaris* infection on the growth of puppies. The phenomena were, to be sure, observed in the course of only one experiment, and certain other factors may have intervened to bring about the results he cites, but they certainly are suggestive. Gibson says:

The existence of an anti-vitamine or at least of growth inhibiting substances formed by ascarids is suggested by an observation which I made in connection with some milk feeding experiments with puppies. In a series of five young puppies fed on cows' milk growth stopped in four of the animals when 44 days old. Following the administration of an efficient vermifuge, there resulted the passage of many ascarids from the four dogs in which growth had ceased. Growth was immediately reëstablished.

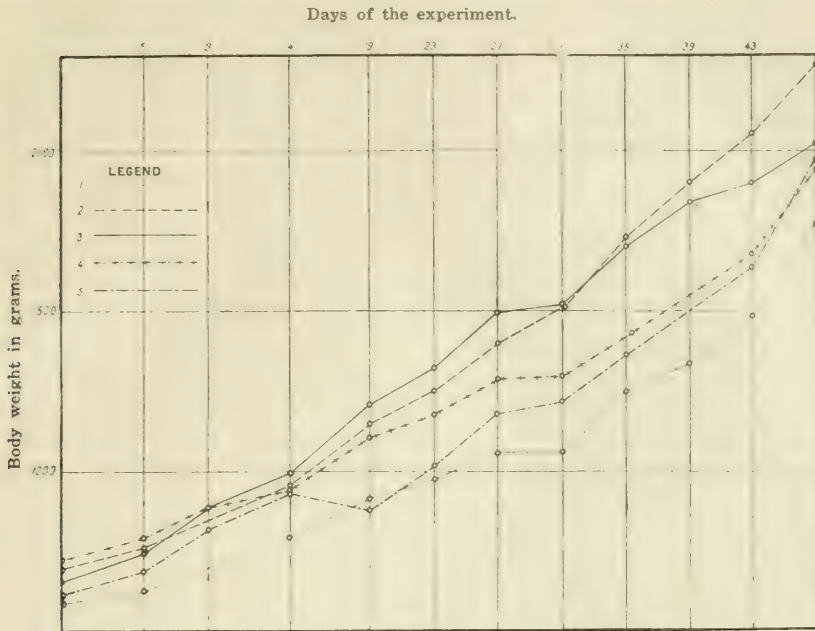


FIG. 1. Growth curves for dogs fed on fresh and autoclaved milk. Note the falling off of growth between the twenty-seventh and the thirty-first day. Note also that dog 2, which was not infected with *Ascaris*, continued to grow at about the normal rate.

The growth chart illustrating this case has been published by Gibson and Concepcion(18) and is reproduced here as fig. 1, by kind permission of the authors.

This is suggestive of the pernicious effects that may occur as a result of heavy infections of protozoa of the so-called "harmless commensal" type, particularly in very young children, and is entirely apart from the menace they afford as possible tissue invaders. It would seem that the end products of protozoan metabolism, in cases where a host is heavily infected, could not fail to affect the host unfavorably, particularly in the upper intestine, where absorption is active. It is known that protozoa in cultures may themselves succumb to the products of their own metabolism, as has been shown by Woodruff(53) in a series of experiments with the free-living infusorian *Paramœcium*. In connection with the suggestion advanced by Gibson, it should be borne in mind that the effects he noted may have simply been those of starvation, through the appropriation by the parasite of food designed for the host, an effect which would probably be particularly noticeable in the case of a young and growing animal.



It has occurred to me that the condition I have mentioned might possibly explain some of the frequent and obscure cases of "auto-intoxication" occurring in the tropics, often under apparently unexplainable conditions; and possibly, also, the case presented by the patient in the tropics who is unable to give his physician any further information than that he experiences a disinclination to work and "feels rotten"—a condition often referred to as "Philippinitis," in the Philippine Islands. I have several times been struck by the fact that a large proportion of such individuals harbored swarms of trichomonads, when there was not a frank infection with helminths, and I have been inclined to suspect that had the opportunity been present for an examination of the stools on several successive days the flagellates would have appeared in the other cases as well. I have noted similar symptoms in individuals suffering from heavy infections with *Spirochæta eurygyrata*.

*Trichomonas* is a protozoan parasite of the order Polymastigida and the subphylum Mastigophora. It is found through a wide range of the lower animals and very frequently in man. It possesses well-developed motile organs and a cytostome for the ingestion of solid food and is, in no sense, to be regarded as a primitive or degenerate species. Its nucleus is of the karyosome type and divides with a well-developed mitotic figure in which chromosomes have been demonstrated in some species. Its life cycle, so far as it has been worked out, seems to be fairly complex. Although the behavior of the chromatin under certain conditions may be regarded as leading up to it, syngamy has not yet been worked out.

The organism shows more or less variation in size and form. *Trichomonas intestinalis* of man measures, generally speaking, from 10 to 15  $\mu$  in length and from 3 to 5  $\mu$  at its greatest breadth. Forms may be found that are almost spherical through those that are triangular, short, or elongated. Many are pyriform or pear-shaped. The organisms are active in their movements, but frequently come to rest, apparently to feed. Their food during life in the lumen of the intestine consists, for the most part, of bacteria. Three equal flagella, inserted in relation to a basal granule or blepharoplast, spring from the anterior end of the body and are employed in locomotion and food-taking. Another, somewhat stouter flagellum runs posteriorly, forming the margin of an extension of the ectoplasm, the undulating membrane, and is continued as a free lash beyond the posterior end. The inner margin of the undulating membrane is bordered by a deeply staining linear struc-



ture, the parabasal body. A clear, refractile, rodlike structure, originating at about the middle third of the body, runs posteriorly and frequently projects beyond the posterior extremity of the body. This is the axostyle. By some authors this is believed to be a stiffening rod for maintaining the contour of the body. Others speak of it as an attaching organ, while Kofoed and Swezy<sup>(26)</sup> state that it is in the nature of a motile organ, or, as they say, "a stout, largely intracytoplasmic flagellum for locomotion in a viscid medium." It has occurred to me that it may, under some circumstances, serve as a piercing organ in connection with tissue penetration. The cytostome is a triangular depression at the anterior end, laterad of the median line. Some writers describe a flagellum within it, an observation I am unable to confirm. The endoplasm contains one or more vacuoles, apparently food vacuoles, for bacteria may be frequently seen within them. I have never observed a contractile vacuole. Excretion may take place by diffusion through the body wall or through the cytostome.

*Trichomonas* is frequently found to bear four or even five anterior flagella, which has caused certain writers to place them in different genera under the euphonious, but somewhat anomalous names *Tetratrichomonas* and *Pentatrichomonas*. I shall not take the time here to discuss the advisability or inadvisability of founding new genera on such trivial variations as these.

Ohira and Noguchi<sup>(40)</sup> have created five subgenera to be included under the genus *Trichomonas* Donné, 1837. They are:

1. *Protrichomonas* Alexeieff, with three anterior flagella, without an undulating membrane.
2. *Trichomastix* Bütschli, with three anterior flagella and a trailing flagellum (*Schleppgeissel*), without an undulating membrane.
3. *Trichomonas* Donné, with three anterior flagella and an undulating membrane.
4. *Macrostoma* Alexeieff, emend. Wenyon, with three anterior flagella, and an undulating membrane wedged in a deep groove (peristome).
5. *Tetratrichomonas* Parisi, with four anterior flagella and an undulating membrane.

To these might be added Chatterjee's *Pentatrichomonas*, with five anterior flagella and an undulating membrane. Other intestinal flagellates of interest here are:

*Lambia* (*Giardia*) *intestinalis* Lambl, 1859. This is an organism of peculiarly striking and characteristic appearance and

one of the few protozoa that are bilaterally symmetrical. The organism is pear-shaped and measures from 10 to 21  $\mu$ , with a width of from 5 to 12  $\mu$ . There is an anterior, oblique depression or sucking disk, the contractile edges of which are raised above the general surface. There is no mouth or cytostome. Two axostyles extend from the region of the sucking disk to the posterior end, where they continue as two stout flagella. There are three other pairs of flagella arising from the borders of the hollow disk, giving eight flagella in all. The organism, as usually seen, is binucleate, the two nuclei of the karyosome type lying within the sucking disk, one on each side of the axostyles. Stained, the organism bears a most grotesque resemblance to a spectacled face. This resemblance extends even to the cysts.

*Enteromonas hominis* da Fonseca, 1915. This organism has been placed in the Tetramitidinae by Chalmers and Pekkola.<sup>2</sup> They describe it as being equipped with three unequal anterior flagella, lacking a permanent cytostome, undulating membrane, and axostyle. The nucleus is of the protokaryon type and is joined to the blepharoplasts by a rhizoplast. This organism they believe to be allied to the genus *Dallengeria* Saville-Kent, 1880. Chalmers and Pekkola regard this organism as pathogenic to man, giving rise to diarrhoea, "and by absorption from the bowel febrile attacks." They record three cases of this infection, the first being reported from Brazil by da Fonseca and the other two in the Anglo-Egyptian Sudan by themselves. They venture the prediction that it may be ultimately found to be widespread throughout the tropics.

*Tetramitus mesnili* Wenyon, 1910. See *Macrostoma*.

*Cercomonas hominis* Davaine, 1854. *Cercomonas* is a very uncertain genus, but a great convenience to those who are unable otherwise to classify an intestinal flagellate. The descriptions vary greatly, and undoubtedly other flagellates such as *Trichomonas*, *Macrostoma*, and the like have been called *Cercomonas* by unskilled observers. *Cercomonas* is described as having a pear-shaped body, 10 to 12  $\mu$  long, which tapers to a point posteriorly. The flagellum is said to be about 20  $\mu$  long. A cytostome has been described by some writers.

*Prowazekia* Hartmann and Chagas, 1910. Species reported as occurring in faeces are: *Prowazekia cruzi*, *Prowazekia asiatica*, *Prowazekia weinbergi*, and *Prowazekia javanensis*. The general characteristics of this genus are the possession of two flagella,

<sup>2</sup> *Bull. Soc. path. exot.* (1917), 10, 756.

one of which is directed forward and the other downward and backward in heteromastigote fashion. There is a principal nucleus (triphonucleus?), and there is another body of supposed nuclear nature that has been variously termed the kinetonucleus, or the blepharoplast. Absolute proof of the parasitic nature of this organism is still lacking, and there is a tendency to regard it as a free-living form, which has contaminated stools or urine. I have never seen it in pond or tap water, but some observers have reported it as occurring free.<sup>3</sup>

To my mind there is some ground for suspecting that *Protrichomonas*, *Trichomastix*, *Macrostoma*, and possibly *Cercomonas* may represent developmental stages in the life cycle of *Trichomonas*, and Minchin<sup>(35)</sup> suggests that as *Trichomonas* and *Trichomastix* frequently occur in the same host they are "perhaps to be interpreted as two developmental phases of the same organism rather than as distinct generic types."

Reproduction in *Trichomonas* has been described by several writers as taking the form of simple longitudinal binary fission, which may, at times, give way to multiple fission. Recent investigations seem to indicate that either form of reproduction may occur. Cyst formation is unproved, many writers doubting if it occurs.

Woodcock<sup>(52)</sup> considers that *Trichomonas* may have lost the power to produce cysts and considers it likely that "*infection with trichomonas can take place by means of the active, unencysted forms.*" (The italics are Woodcock's.) He found that *Trichomonas* would live and remain active for five and one-half hours, both in 0.066 hydrochloric acid solution and also in pancreatic solution of a strength that would bring about the excystation of *Entamæba histolytica*.

In this connection Wenyon<sup>(50)</sup> states that the rounded-out forms are capable of resisting the action of gastric juice for a considerable time, which might, as Woodcock says, account for the safe passage of the naked parasites through the stomach. The bodies appearing in the fæces of man and of some of the lower animals and generally spoken of as *Blastocystis enterocola* or *Blastocystis hominis* have been thought by many to be

\* Macfie (*Journ. Trop. Med. & Hyg.* (1917), 20, 1), reporting from Accra, states that flagellates soon make their appearance in bottles of saline solution or distilled water exposed in the laboratory. Apparently the organisms develop in the thin layer of fluid between the neck of the bottle and the ground glass stopper. They are not usually found in the fluids inside the bottles. These flagellates appeared to belong to the genus *Prowazekia* of Hartmann and Chagas.



the cysts of *Trichomonas*, but as will be shown this remains to be proved. Prowazek, (43) following a study of them, has declared his belief that they are the cysts of a flagellate, *Bodo lacertæ*. Added evidence of their flagellate nature has been recently produced by Chatton, (9) and further presumptive evidence is gained by a study of the plate illustrating Hadley's paper. (20)

A review of some of the clinical observations on flagellate infections discloses a variety of opinions, which coincide somewhat with the views that have been held regarding the etiology of *Entamæba histolytica* in dysentery. Some writers frankly state their belief that *Trichomonas* and some of the other flagellates are pathogenic and that they may give rise to the symptom complex known as dysentery. Others believe that they will produce nothing worse than diarrhœa, while still others adopt an intermediate position in holding that while incapable of initiating lesions they may aggravate preëxisting lesions. Brumpt (4) has described a case of colitis in a patient returned to France from Tonkin that he ascribes to infection with *Trichomonas intestinalis*. Escomel (11) has written of one hundred fifty-two cases he speaks of as dysentery, occurring in Peru. The causative organism he believes to have been *Trichomonas* and claims that the source of infection was traced to a polluted water supply. Mello-Leitao (31) traced cases of dysentery in children in Rio de Janeiro to *Trichomonas intestinalis* and *Lambliã intestinalis*, occurring either separately or together. He believes that flagellate dysentery is benign, but that the organisms may be pathogenic to children under 3 years of age. This form of dysentery, he thinks, is the most frequent type occurring in infants. Derrieu and Raynaud (10) report a case of chronic dysentery in Algiers, which they lay at the door of *Pentatrichomonas bengalensis* Chatterjee, 1915. In the United States, Rhamy and Metts (44) state their strong belief in the pathogenicity of *Trichomonas intestinalis* and give detailed clinical reports of several cases. The senior author says that in an experience extending over seventeen years he has never found flagellated protozoa in stools except in cases with existing or recent acute or chronic diarrhœa. He states his belief that sufficient importance has not been placed on the pathogenicity of this parasite.

Rhamy and Metts review an epidemic of flagellate dysentery involving seventy-eight cases with seventeen deaths and they also present seven selected cases of their own, none of which, however, went to autopsy. These cases they characterize as "dysenteric diarrhœa." They state that so far as they could



ascertain the disturbances were caused by the flagellated protozoa. They go on to say, however, that cases recovered under antidyenteric treatment with ipecac and its compounds. This appears to be at variance with the experience of other workers, who almost unanimously report unsatisfactory results in the treatment of flagellate infections with ipecac and raises the question of the possibility that the authors might have overlooked *Entamæba histolytica* in their examination of the stools. Apparently bacillary dysentery was not absolutely ruled out. The cases they trace to impure water.

These authors describe the symptoms as consisting of diarrhœa with colicky pains, watery or slimy blood-stained stools, weakness, dyspnœa, loss of weight, progressive anæmia simulating pernicious anæmia, and the skin appearing yellow with urticarial or pellagroid eruptions. Later the stools consisted of mucus, blood, pus, and active trichomonads. The blood showed a moderate eosinophilia (6 to 12 per cent). Proctoscopic examination of one of their cases showed the presence of a large shallow ulcer on the posterior wall of the rectum. The remainder of the rectum was covered with mucus and was congested. The stools of this patient contained blood, pus, and trichomonads.

A complete description is not given of the organisms seen by these authors; that is to say, the description is not sufficiently complete to leave their identity as *Trichomonas* beyond question, and indeed, such is the case with many of the reports that are available to me. This makes it difficult in some instances to decide what flagellate is involved.

Of interest in connection with this observation is a case I recently saw in consultation with Dr. A. F. Coutant, at St. Luke's Hospital, Manila. The patient was a young American woman, married, who some time previous had suffered an acute attack of intestinal entamœbiasis. She came to the hospital for treatment of a persistent diarrhœa, which, though troublesome, did not prevent her from going about. Repeated examination of the stools of this patient failed to disclose the presence of entamœbæ, but the stools contained considerable mucus and an occasional red blood corpuscle. Eventually *Trichomonas intestinalis* was found in small numbers. Proctoscopic examination of the patient disclosed a small eroded area on the wall of the rectum. This spot was not ulcerated. A small mass of mucus was carefully removed from this eroded area with a sterile platinum loop. Microscopic examination of this mucus revealed a large number of active trichomonads. Under treatment with

methylen blue the trichomonads disappeared and with them the diarrhoea. Soon afterward the patient left for the United States, and the case passed out of observation.

Prentiss<sup>(42)</sup> reports several cases of infection with an organism he describes as *Cercomonas hominis*. He describes it as a "flagellated protozoön, bluntly pointed at both extremities, with a thin flagellum at one end, which by waving actively imparts motion to the organism similar to that of a tadpole." This organism he has found in the fæces of a number of his patients in the southwestern United States (he writes from El Paso, Texas). This parasite was found in the fæces of patients having diarrhoea—some chronic, others acute. *Entamæba histolytica* was not found. Some of the cases assumed a severe form, and two terminated fatally with symptoms suggestive more of an acute catarrh than of ulceration. In one fatal case that was necropsied, the intestine showed no trace of ulceration. The author says no other cause of death could be found. The identity of the organism seems to be in doubt here.

Chatterjee,<sup>(8)</sup> however, is more explicit. He boldly designates flagellate dysentery as "a distinct entity." He studied seventy cases in India and tabulates his results in Table I as follows:

TABLE I.

Organism.	Character of stool.	Number of cases observed.
<i>Monocercomonas</i> .....	Choleraic .....	3
<i>Prowazekia</i> .....	do .....	2
<i>Macrostoma</i> .....	Chronic dysentery .....	18
<i>Lamblia</i> .....	do .....	15
<i>Pentatrichomonas</i> .....	do .....	32

He concludes that *Macrostoma*, *Lamblia*, and *Pentatrichomonas* cause intestinal trouble. *Monocercomonas* and *Prowazekia* he regards as harmless, pointing out their occurrence in cholera motions. To this latter statement I may add, as I have already said, that the systematic position of the cercomonads is uncertain,<sup>4</sup> and *Prowazekia* is thought by many to occur as a contamination of fæces or urine and not to be a parasite.

Nearly every writer on tropical medicine has spoken of diarrhoea as a concomitant of flagellate infection. Musgrave<sup>(38)</sup>

<sup>4</sup> *Monocercomonas*, according to Alexeieff, has four anterior flagella. Sometimes these are of equal length, or two may be shorter and two longer.

has given voice to the belief of the early workers in Manila that many of these so-called harmless parasites are disease producers. He adds that several of the Manila men (he wrote in 1906) recognized a diarrhœa caused by "monads." He says:

Several types of these parasites, when present in large numbers, are very intimately connected with chronic diarrhœa and they are surely much more important than they are generally considered to be. [p. 561.]

He views *Lamblia intestinalis* with particular suspicion and goes on to say that "when encountered in great numbers, it is *always* associated with chronic diarrhœa, which disappears with the destruction of the parasites."

With *Lamblia* the conditions to me seem to be more obvious, for here we have a parasite that we know attaches itself to the epithelium by its sucking disk. It seems reasonable to conclude that this alone would give rise to considerable irritation, to say nothing of the facility with which toxic excretory products of the parasite could be absorbed by the epithelium with which it is in such close relation. In the cases of the other flagellated protozoa we have less information on that point. Musgrave's belief is that *Lamblia* "bears a decided causative relation to the diarrhœa."

Speaking of the intestinal parasites in general, including the helminths, Musgrave concludes that—

Whatever pathological significance may be attached to these parasites in general, some of them, particularly the actively motile ones, such as monads, surely aggravate amœbic ulcers in which they may be present.

Fantham, Stephens, and Theobald<sup>(14)</sup> say that "like *Trichomonas*, *Lamblia* can multiply under inflammatory conditions of the alimentary tract."

Fantham and Porter,<sup>(12)</sup> following a series of carefully conducted experiments, reached the conclusion that *Lamblia* is pathogenic to man and is capable of producing diarrhœa, which may be persistent or recurrent. Furthermore they hold the view, which to me is of great importance in considering the relations of the intestinal flagellates to man, that the virulence of the parasite varies and lambliasis occurs in tropical and nontropical countries. *Lamblia* cysts, they say, will remain infective for some time.

Whatever the case, the flagellates certainly often persist in the stools for long periods of time, and diarrhœa is the rule when they are present in large numbers. Whether they cause the diarrhœa or whether the increase in their number is brought about by the diarrhœa is quite another question. The most



charitable attitude from the viewpoint of *Trichomonas* is taken by Minchin<sup>(33)</sup> who says:

The common intestinal flagellates belonging to the genus *Trichomonas* and other genera are \* \* \* not to be regarded as true parasites in any sense of the word \* \* \* Many of these intestinal Protozoa are perhaps useful, rather than harmful to their host.

At least four authors report infection with *Trichomonas* through the drinking of impure water, and in at least two cases there were epidemic outbreaks alleged to be due to *Trichomonas*. Smithies<sup>(47)</sup> reports two cases of severe dyspepsia in which he recovered *Trichomonas* from the stomach. These cases occurred in the southern United States. The infection in one case he attributes to the drinking of unfiltered surface water by the patient. In the epidemic in Peru, reported by Escomel,<sup>(11)</sup> which I have already mentioned, he states that examination of the reservoirs containing the water used for drinking purposes showed the presence of *Trichomonas*. When the reservoirs were cleaned, the organisms disappeared, and the outbreak ceased. As will be shown later on, *Trichomonas* has been cultivated by at least two groups of workers, and it is a fact well known to all tropical workers that the parasites will survive in stools for many days. Kofoed and Swezy<sup>(26)</sup> made cover glass preparations of *Trichomonas augusta*, which they diluted with normal salt solution and sealed with vaseline. They report that the parasites were "kept alive for several months without any change in the medium, or removal of the cover glass." I have had the same experience with *Trichomonas lacertæ*, which I have kept in mounts sealed with paraffin, the organisms continuing to live in physiological salt solution for upward of six weeks. At the end of that time the sealing of the mounts became loosened, and the preparations dried with consequent death of the organisms. Lastly there is the epidemic of trichomoniasis reported by Rhamy and Metts,<sup>(44)</sup> as involving seventy-eight patients at Liberty Township, Indiana, in 1909.

Of course, in all these cases possible mistakes in the identity of the forms mentioned must be borne in mind. While undulating membranes of the type seen in *Trypanosoma* and *Trichomonas* are characteristic of the parasitic species, still there remains the possibility of mistaking some of the multiflagellate species of free-living protozoa for *Trichomonas* and other intestinal flagellates.

I might, in passing, remark that I have received, on more than one occasion, contaminated stools or urine that have contained undoubted fresh-water forms such as *Phacus*, *Arcella*, and *Pera-*



*nema*, and have been asked to identify these new intestinal or vesical parasites. Unfamiliarity with free-living species has undoubtedly led to the discovery of "new protozoan parasites" on some occasions. This should not, however, be construed either as a reflection on the powers of observation of these authors or a denial of the possibility that *Trichomonas* may be capable of living in pond water. Like *Balantidium*, its food-getting apparatus is adequate, and it is likely that it could secure its proper food in sewage-polluted waters. But it remains to demonstrate the fact experimentally one way or the other or by the observation of the organisms in pond water.

There was an outbreak of diarrhœa at Parañaque, a suburb of Manila, in 1914. The local sanitary inspector at that place assured me at the time that the diarrhœa was epidemic in the town and that there had been a large number of cases, some of which had been attended with fatal results. He stated his belief that the trouble was due to *Trichomonas*, with which he thought the sufferers had been infected through drinking river water. He had no material at hand that I could study, and pressure of other duties made it impossible for me to investigate the matter further.

Fantham, Stephens, and Theobald<sup>(13)</sup> believe that air, water, and on some occasions food may be vectors of *Trichomonas*.

As for *Lambli*a, Mathis<sup>(30)</sup> and Fantham and Porter<sup>(12)</sup> say rats are transmitters and reservoirs for the species infesting man. A similar condition may exist in the case of *Trichomonas* and the other intestinal flagellates. It is pretty well established that pigs are reservoirs and transmitters of *Balantidium coli*, and the rat is under suspicion in connection with entamœbiasis.

Unfortunately Hadley<sup>(20)</sup> does not figure the intracellular stages of *Trichomonas* in his cases of blackhead in turkeys, and we shall have to await his future communications for these. It would be interesting to see how closely his intercellular parasites coincide in appearance with *Entamœba histolytica* as the latter appear in sections through the intestine. Shorn of its flagella, undulating membrane, and axostyle and possessing a nucleus of the karyosome type characteristic of the entamœbæ, it is not hard to see how *Trichomonas* could have been mistaken for *Entamœba* even by careful workers.

Hadley speaks of the trophozoite stage in the lumen of the intestine, as the period of youth during which the organism divides by simple, longitudinal fission and accumulates a reserve of food substance in the endoplasm. When this food reserve has accumulated to a sufficient amount, this method of reproduc-

tion gives way to what Hadley styles a form of "autogamous reproduction." This he has not worked out in all its details, and I am unable to determine if it is autogamy or merely multiple division within a cyst, although he mentions the casting off of nuclear material, which might be interpreted as a process of nuclear reduction. The fusion of gametic nuclei, such as has been described by Wenyon<sup>(51)</sup> for *Entamoeba muris*, is not mentioned by Hadley, but he speaks of the possible conjugation (copulation?) of flagellates derived from the original trophozoite. These flagellates might be in the nature of swarmers produced after the reduction process spoken of by Hadley, or they might be trophozoites undergoing copulation as has been described by Dobell in the case of *Copromonas subtilis*. Schaudinn in short note,<sup>(45)</sup> has stated that *Trichomonas* becomes an amoeba and that two of these amoebæ, after giving off reduction nuclei, encyst together and carry out syngamy within the cyst. Later the zygote breaks up, forming small individuals, leaving a mass of residual protoplasm behind. However, this has not been confirmed, and indeed the whole process of syngamy in *Trichomonas* remains to be worked out, as does the same problem in the larger number of intestinal protozoan parasites.

In the process described by Hadley, the trophozoite usually increases in size, rounds off, and secretes a cyst. The parabasal body lengthens, until it forms almost a complete circle near the periphery. The flagella, undulating membrane, and cytostome are gradually lost, and the vacuole or vacuoles of the trophozoite coalesce and enlarge until the single vacuole occupies the greater portion of the ventral part of the animal. The cytoplasm and nucleus are flattened against the dorsal wall. The chromatinic blocks and axostyle degenerate and disappear. The parabasal may persist with the blepharoplast for a time, but eventually they, too, disappear.

At this stage the vacuole, which is held to contain the store of reserve food, has increased in size, until it occupies the larger part of the cell and is surrounded by a crescentic ring of cytoplasm, which seemingly has become much reduced in amount. The nucleus is flattened or flask-shaped and divides, the daughter nuclei taking up positions at opposite sides of the food vacuole. At this stage the organism certainly bears a striking resemblance to *Blastocystis* and to the figures in Chatton's recent paper. The nuclei may then divide to form four, eight, or sixteen daughter nuclei, which arrange themselves about the periphery.

Hadley mentions at this point that smaller portions of nuclear

substance may be seen in the cytoplasm following the first nuclear division and states his belief that they represent the reduction bodies I have already mentioned. He has not observed them in the process of formation.

Following the multiple division of the nucleus, cytoplasm collects around each nucleus, and the small cells thus formed break from the peripheral ring and enter the centrally situated food vacuole, which would then appear to become something in the nature of a brood chamber. The substance within this vacuole seems to be gradually consumed by the cells, which grow in size. Next the cyst wall weakens, the young organisms burst out and swim off as trophozoites, measuring 4 to 5  $\mu$  in length by 3  $\mu$  in width, each equipped with an anterior flagellum, a nucleus, and a blepharoplast. Gradually these young forms develop additional flagella and the other organelles characteristic of *Trichomonas*. During this series of developmental changes it would appear that the organism might come to resemble Wenyon's *Macrostoma mesnili*.

It is at this stage that Hadley says he has evidence of conjugation between two or three or even four individuals. This conjugation of more than two individuals would constitute something of a departure from the process usually observed in the Protozoa where syngamy occurs (when it is not autogamous) between two individuals only. It is hard not to regard the union of three or even four individuals under such circumstances as being wholly fortuitous and probably unproductive of results. At periods of sexual maturity in the Protozoa it is not infrequently observed that the ectoplasm of the organisms becomes sticky, so that they have a tendency to adhere in pairs if they blunder against each other. This is strikingly seen in the conjugation of *Paramaecium caudatum*; and it is not very unusual when epidemics of conjugation occur in a culture, as they frequently do, to see three or four irregularly attached individuals swimming about in a clump. Usually the matter adjusts itself with the aid of the ciliary currents of the animals, which tend to bring them into the proper position for carrying out the process of conjugation.

If conjugation or copulation of these flagellated individuals takes place, as Hadley suspects may be the case, it seems to be a process of either endogamy or exogamy, depending, of course, upon whether union took place between cells derived from the same or from different cysts. In that case Hadley's autogamy would fall and the process of nuclear reduction he suggests



might be, as I have already said, a reduction process preliminary to the formation of flagellated gametes.<sup>5</sup>

Following the copulation of these flagellated cells, Hadley mentions the formation of a viscid membrane around the organisms, which have meanwhile rounded off. This might coincide with the process described by Schaudinn<sup>(45)</sup> mentioned previously. Following conjugation, adds Hadley, the membrane hardens to form a firm cyst. The single cysts, he says, measure from 10 to 12  $\mu$ , but in the "fused" or conjugated forms the cysts may reach a size of 20 to 30  $\mu$ . "Double" or "triple" cysts, he concludes, may represent a division of the original cysts, whereupon each cyst continues to produce daughter cells independently.

This stage of the life cycle, according to Hadley, is the one usually encountered and which has caused *Trichomonas* to be regarded as a harmless commensal; but he goes on to show that in another phase of activity the parasite may penetrate the epithelium and cause fatal lesions in the intestinal tract. The question is, what stimulus is it that causes the organism so radically to alter its mode of life. To my mind this very question is to-day one of the most important problems of parasitology.

In the turkeys observed by Hadley the invasion was invariably preceded by diarrhoea with an accompanying increase in the number of parasites as the disease advanced. The parasites appear not only in the liquid caecal contents, but in the depths of the caecal tubules or crypts and finally in the tissues behind the epithelial walls. Hadley states that by a process of "auto-gamous reproduction" the mucosa, submucosa, and muscularis mucosae and even the muscular layers are successively invaded, until the whole caecal wall is involved. Secondary bacterial invasions may supervene to bring about results that are almost invariably fatal. Here Hadley raises the old question as to whether the vast numbers of parasites present are the cause or the result of the diarrhoea. This point will be further discussed.

<sup>5</sup> In a recent letter to me, Doctor Hadley says: "Regarding the presence of syngamy in the reproductive process, I doubt very much that it occurs in the flagellated swimmers. I have never seen the least suggestion of it. I am rather of the opinion that, when it occurs at all, it takes place in the stage after the organism has lost its chief features of flagellate morphology, has become globoid and possesses a more or less viscid capsule or membrane. In fresh preparations such appearances, at least in the first adhesive stages, are fairly common." It is a matter of great regret to me that three other papers by Doctor Hadley, dealing with this subject, reached me too late for consideration in connection with this paper. They should be consulted by all who are interested in the subject. [See *Bull. Agr. Exp. Station*, Rhode Island State College, Kingston, R. I. (1916), Nos. 166 and 168, and *Journ. Med. Res.* (1917), 36, 79.]



Apparently the parasites gain entrance to the tissues through the goblet cells of the crypts of Lieberkühn. They accumulate in large numbers in the fundi of the crypts, causing the walls to bulge, with the result that the parasites are literally forced into the goblet cells, past the nucleus and cell wall, until finally they enter beneath the epithelium of the crypt. The passage once made, other trichomonads follow and collect in a mass between the epithelium and the basement membrane.

This tendency to collect about the surface of the epithelium has been noted by other workers. Kofoid and Swezy<sup>(26)</sup> and Martin and Robertson<sup>(29)</sup> mention it in connection with their search for dividing forms.

Next the parasites push through the basement membrane into the connective tissue of the mucosa and thence to the muscularis mucosæ and submucosa. The invasion is described as being of an intracellular nature as in the case of the Sporozoa.

Smith<sup>(46)</sup> has discussed at length some peculiar bodies he found in 1916 in a turkey suffering supposedly from blackhead, which he was inclined at the time to regard as coccidia that had wandered beyond their accustomed habitat, the epithelium. Characteristic lesions of blackhead were absent, and several coccidial cysts were found in the fæces. The article is too long to quote in extenso, but the author mentions certain things that may have a bearing on Hadley's observations. Sections of the intestinal tract showed that the epithelium had been lifted from the core of the villus, leaving the space intervening filled with a precipitate of fine granules. The parasites appeared as an almost continuous band near the margin of the villus core. The striking feature seems to be the appearance of these bodies, which were vacuolelike and partially empty. A few were filled. Smith says:

They consisted of some host cell whose cytoplasm had been moulded into a shell (or ring in section) with the much flattened nucleus against this shell. The contents were a very fine lining membrane within which were roundish bodies of various diameters  $2\ \mu$  and more, staining feebly reddish and with or without a mass of chromatin. Frequently a body contained two chromatin masses situated at opposite poles, as if division had taken place. Those bodies which were full of spheres, contained about sixteen or more of more or less uniform size. The vacuolated appearance under low power was due to the disappearance of some or all of the parasitic contents of the host cell. Prolonged search for the characteristic products of asexual multiplication—falciform bodies—brought to light only two or three parasites containing them. It is not to be denied that these may have been moulded into crescent shape by the pressure of the other growing and segmenting members in the same membrane.

Were these bodies trichomonads undergoing Hadley's autogamous reproduction in the tissues? Undoubtedly the turkey that formed the material for Smith's study was infected with *Coccidium*, and parasites found in the epithelial cells were, according to Smith, clearly coccidia. It was the collection of bodies in the subepithelial tissues that was regarded by him as representing something different. However, he considered them to be aberrant coccidia, and the possibility of their being trichomonads is not discussed by him.

Going back to Hadley's account of *Trichomonas*, we note that this author mentions the engulfing of the flagellates by endothelial and other phagocytic cells. This, in many cases, seems to have had no untoward effect upon the parasites, which showed a marked resistance to the phagocytes and even seemed to divide within them. Instances in which infection with microorganisms is spread by phagocytes are known. *Hepatozoön perniciosum*, a sporozoan parasite of the rat, has been described by Miller<sup>(32)</sup> as passing a portion of its life cycle in encysted form in the large lymphocytes of the rat, and protozoan parasites of leucocytes have been long known. Hadley believes that the protozoa in his case use the host phagocyte as food and that they may, indeed, fare better within than without the phagocytic cells. In the regions of the invaded tissue he has found the majority of the parasites present in phagocytic cells, which he cites as proof of the rôle the phagocytes play in spreading the infection.

In the lumen of the intestine the trichomonads are holozoic forms, subsisting largely on the bacteria found there. In the tissues their morphology is markedly altered, and the evidence, according to Hadley, indicates the substitution of an osmotic method of nutrition. A point is raised here as to whether or not all tissue-dwelling forms are nourished by absorption or whether such forms as *Entamœba* and *Balantidium* do not continue to nourish themselves by the holozoic method. If, in the tissues, *Trichomonas* becomes virtually an amœba, does it take in solid food or does it derive its support from fluid substance absorbed through the body wall?

Hadley states that although the motile forms can be recognized in the tissues, as can some of the sporulating forms, these are relatively scarce, and the stage that shows the well-rounded ball of reserve food substance, which appears to be in the nature of glycogen, is seldom encountered. Further details as to the appearance of these parasites in the tissues are necessary before any final conclusions can be drawn regarding the presence of an altered form of *Trichomonas* in the human intestine.

The author (Hadley) points out the obvious difficulty of escape from the hosts that these parasites buried deep in the tissues would meet; but again we may find instances of this very contingency in the literature on the Protozoa, where the death of the host is necessary before the spores can be disseminated. However, he shows how the parasites may return to the lumen of the cæcum by spreading downward and inward through the cores of the villi, finally breaking through the epithelium by sheer pressure of numbers, much as they made their entrance.

Kofoid and Swezy, (26) in their studies of mitosis and multiple fission in the trichomonads, bring out several points that are of interest in connection with the work of Hadley. They state their inability to present conclusive evidence "that either leads to gamete formation by maturation divisions, or that either follows zygote formation or fertilization." This knowledge they believe will come only with the solution of the history of the "true trichomonad cysts."

There is a great diversity of opinion as to whether the encysted stage of *Trichomonas* or some other flagellate is represented by *Blastocystis*. Wenyon, (50) while admitting that on occasions it has seemed to him that degenerating *Trichomonas* or *Tetramitus* may become centrally vacuolated and resemble *Blastocystis*, thinks it is untenable to view the latter as encysted *Trichomonas*. Kofoid and Swezy are skeptical of the relation of *Blastocystis* to *Trichomonas* and figure *Trichomonas* with an engulfed cyst of *Blastocystis enterocola*. However, that does not necessarily constitute a conclusive argument, for I have seen *Vahlkampia* engulf cysts of its own species. Galli-Valerio (15) found double-contoured cysts in the fæces of guinea pigs infected with *Trichomonas*, after the fæces had been kept in a damp chamber for one month. When warmed, the cysts opened and discharged small flagellates. Administration of the cysts caused infections in other animals. Alexeieff (1) and Brumpt (4) doubt encystment in *Trichomonas* and state their belief that these cysts (*Blastocystis*) represent a stage in the life cycle of some fungoid or yeast organism.

Swellengrebel<sup>c</sup> has reviewed the entire controversy and, following an investigation, reaches these conclusions:

1. In two cases *Blastocystis* was found where the presence of *Trichomonas* or *Chilomastix* could be excluded with absolute certainty. Consequently *Blastocystis* cannot be a normal developmental form of either.
2. In fresh stools *Blastocystis* is but seldom found to be alive and even

<sup>c</sup> *Parasit.* (1917), 9, 451.



when encountered in this state it dies quickly. After death the central sphere soon disappears.

3. The size of *Blastocystis* varies greatly and the larger they grow the smaller becomes the peripheral fringe of cytoplasm. Living blastocysts are relatively small and rich in cytoplasm.

4. The blastocysts of the cases mentioned here, although having some general characters in common differed much as to details of structure. This difference was especially marked when the associated parasites were different. No blastocysts were found without an associated parasite.

5. The occurrence of blastocysts in the stools of a man fed on milk and eggs only, and the presence of living blastocysts in the man's stools, exclude the idea of their being remains of solid food.

6. It is probable from the observation recorded in this paper, that "Blastocystis" is not the name of a zoölogical genus but of a peculiar form of degeneration to which representatives of different genera of intestinal protozoa may be liable. The resemblance seen in blastocysts from different sources which may lead to their being regarded as belonging to one species is easily explained by a convergence resulting from the parasites which produce blastocysts losing their characteristics during the process of degeneration.

7. No certain stages of sporulation were seen as described by Alexeieff, and the nuclear structure, although variable, never resembled that given in his description. It is therefore probable that Alexeieff's *Blastocystis enterocola* is different from the form described in man under the same name.

Chatton (9) also has done some recent work on *Blastocystis*, and while his preliminary paper does not directly bear on *Trichomonas*, his account, coupled with his figures, are very suggestive in connection with the work of Hadley, Kofoid and Swezy, and others. His work is largely confirmatory of that of Prowazek<sup>(43)</sup> on *Bodo* (*Heteromita*) *lacertæ*, but the host studied by Chatton was the Barbary gecko, *Tarentola mauritanica*. The appearances he notes are similar to those mentioned by other writers. He failed to secure a glycogen reaction in the vacuole whose contents he assumes to be of protein nature. Alexeieff laid some stress on the presence of glycogen in determining the bodies to be of blastomycetic nature, but it must be borne in mind that the cysts of *Entamæba* at times contain glycogen. Chatton describes the formation of flagellated cells within the microspheres. He has also seen the coupling of these flagellates, a performance probably similar to that described by Hadley, but he did not see subsequent fusion. Therefore he thinks the existence of copulation at this stage very probable.

Turning to trichomonad infections in man, there seem, in view of the foregoing, to be at least two important observations: namely, those of Castellani on *Entamæba undulans*<sup>(7)</sup> and of Gauducheau on the unity of *Trichomonas* and *Entamæba*.<sup>(16)</sup>



This again gives rise to the question as to whether *Trichomonas* as it appears in the intestinal tissues of man, if it does, so closely resembles *Entamoeba* as to have been mistaken for the latter by the skilled observers who for years have been studying sections of the human intestine taken from cases of dysentery. Hadley's sections of the turkey cæcum ought to throw considerable light on this matter.

Castellani described his organism in 1905 as *Löschia undulans*. Gauducheau has reported a similar organism in the fæces of dogs. Wenyon<sup>(50)</sup> has already called attention to the similarity that *Trichomonas* bears to *Entamoeba undulans* when the former is casting off its motile apparatus and assumes the amœboid form. The case reported by Castellani was in a European planter of Ceylon, who gave a history of entamœbiasis of the intestine and liver. His stool contained, in addition to the organism described by Castellani, *Entamoeba* and *Trichomonas*. The organism measured 12 to 30  $\mu$ , but smaller forms were occasionally encountered. They had no flagella. There was a distinct undulating membrane along one border. Long, straight, finger-formed pseudopodia were rapidly extended and retracted, one at a time. No ecto- or endoplasmic differentiation was noted. The cytoplasm was finely granular and contained bacteria and a noncontractile vacuole.

There now seems little doubt that Castellani's *Entamoeba undulans* is *Trichomonas*. The appearances described by him coincide so closely with developmental changes that have been seen in *Trichomonas* as practically to banish doubt on this point.

In the general consideration of these matters it seems worth while in passing to mention briefly Ijima's *Amœba miurai*, described by him<sup>(23)</sup> and by Miura.<sup>(37)</sup> It is not by any means certain that the bodies described by these two Japanese workers were living organisms. Many writers voice the opinion that they were tissue cells present in a serous exudate. In any event, the bodies were spherical or ellipsoidal. One end bore a small protruberance from which sprang several filamentous processes described as pseudopodia, but which more closely resembled cilia. The whole body measured from 15 to 38  $\mu$ . The cytoplasm was granular with no ecto- or endoplasmic differentiation. It contained several vacuoles, none of which was contractile. One to three nuclei were demonstrated on the addition of acetic acid. The bodies were discovered in the serous fluid of a young woman, who had died of pleuritis and peritonitis endotheliomatosa. Similar forms appeared two days before death in the bloody stool of the patient. Amœboid motion was not noted.

Gauducheau,<sup>(16)</sup> in 1912, announced his belief in the identity of *Entamoeba* and *Trichomonas*. His observations apparently were made on an organism similar to that described in 1907 by Billet under the name *Trichomonas dysenteriae*. Gauducheau claims to have isolated and cultivated *Entamoeba* from a case of dysentery in man. He describes the organism as reproducing by multiple budding and giving rise to large, branched plasmodia. Cultivation he found difficult on nutritive agar sown with cultures of the *Bacillus coli* group. The organisms gave rise to spirochaete-like bodies in the culture. The ectoplasm was clear and formed pseudopodia resembling an undulating membrane, and endogenous buds were formed within the amoeba. In the intestines of man and dogs, which had been injected with cultures of these amoebae, Gauducheau reported finding all the intermediate stages between *Entamoeba* and the flagellate. In the human bowel he found branched plasmodia from which flagella protruded in a manner similar to the appearance in his cultures. The flagellate, he states, develops to an amoeba, and therefore he concludes the identity of *Entamoeba* and *Trichomonas*. This caused him to fix three stages in the cycle of the parasite: 1, a cycle in the tissues of the host, the pathogenic phase; 2, a stage of saprophytism in the lumen of the bowel or in cultures during which time it lives on bacteria; 3, a stage when the completely developed organism lives free. He links Castellani's *Entamoeba undulans* to this organism.

Mention has been made of the significance of diarrhoea in flagellate infection. Hadley and many others have noted the increase in numbers of the parasites under diarrhoeal conditions. The doubt has lain as to whether the flagellates are the cause of the diarrhoea or whether the diarrhoea brings about an increase in their numbers. If the latter supposition is true, we have two possibilities: namely, either the change in composition of the faeces, through affording a more favorable environment in general, or through providing an increased food supply, accelerates division; or else the action of the diarrhoea is merely mechanical, tending to flush the flagellates out of the folds of the intestine. Barlow<sup>(2)</sup> found that twenty-five of his one hundred cases showed the presence of trichomonads in the stools after the administration of a purge. Of these cases, twenty-two had been reported negative on the routine examination of their stools. Alternate diarrhoea and constipation were present in fourteen cases, and in six of the latter there were other conditions adequate to explain their symptoms. Five cases showed

the presence of *Entamæba histolytica*, and three others were positive for an *Entamæba* of an undetermined species.

Barlow found that indiscretions of diet or the administration of cathartics would invariably cause the appearance of the parasites, and he concluded that the increase in numbers was the result and not a cause of the diarrhœa, and that the augmentation was dependent upon more favorable conditions for growth provided by the fluid stools. His study was based on *Trichomonas*, but it apparently was of the tetratrachomonad type, for he described his organism as having four flagella. As he was unable to demonstrate the axostyle in the examination of several hundred specimens, it is barely possible he was dealing with *Macrostoma* or some other species.

Woodcock<sup>(52)</sup> believes that *Trichomonas*, *Lambliæ*, and *Balanitidium*, like *Entamæba histolytica*, may occur without causing symptoms, "but on the other hand they are *potentially* harmful." He describes trichomonad stools as loose or thin diarrhœal, containing sometimes a little mucus and scattered pus cells. In a few of his cases manifestly dysenteric stools contained the parasites. The great majority of the stools were never of a dysenteric character, these being cases of pure *Trichomonas* infection. The diarrhœa, however, was troublesome and chronic and often resisted all treatment.

Castellani,<sup>(6)</sup> discussing the intestinal flagellates as a group, thinks that when present in small numbers they are probably harmless, but in large numbers they may give rise to symptoms. *Lambliæ* he regards as the most pathogenic of all. The patient, he says, complains of the diarrhœa, and the yellowish stools may contain a little mucus. He has never found blood nor pus.

Wenyon<sup>(50)</sup> is another worker who states his belief that diarrhœa favors the multiplication of intestinal flagellates. Personally I have often seen them in formed as well as in diarrhœal stools, sometimes associated with *Blastocystis* or entamœbic infections, but more often not. I have seen only two pure infections with *Blastocystis*.

All this raises the question as to how the reactions of the environment affect the mode of life of the parasite in regard to nutrition and reproduction. Protozoölogists are familiar with the remarkable powers of adaptation shown by both the free-living and the parasitic Protozoa. The changes in mode of life and reproduction that follow the transfer of the malarial parasite from the warm environment of the human blood stream to the cold and otherwise different environment of the gut of



*Anopheles* are familiar. Similar differences are seen in the case of *Trypanosoma lewisi* as it lives in the rat and as it lives in *Ceratophyllus*. There are countless other examples, such as that afforded by *Euglena* on its removal from the influence of sunlight to darkness. Is it, therefore, wholly improbable that changes may occur in the intestinal tract that may lead to the substitution of a cytozoic or histozoic life for a coelozoic life in the case of the intestinal parasites? *Balantidium* and even *Entamoeba*, it is well known, may live in the intestinal tract for long periods of time without giving rise to symptoms, yet sooner or later they may invade the tissues. What are the conditions that lead to these diverse modes of life in the same organism living in the same site? May we not rather seek the answer in the host than in the parasite directly, and is it not something more than a mere lowering of vitality? Is there any good reason why changed conditions may not substitute tissue parasitism for lumen commensalism in the case of the flagellates? It seems improbable that the reactions of immunity as recognized in connection with bacterial and allied infections are to be considered here, for immunity as we know it in the Protozoa seems to be of an exceedingly low order.<sup>7</sup> Does it not seem that we are dealing with chemical affinities of a different nature, chemical reactions governing the regulation of the diseases of a characteristically nonfebrile character unaccompanied by phenomena of immunity? These phenomena need not be necessarily restricted to the metabolic chemistry of the parasite. They might be

<sup>7</sup> In connection with immunity problems with the Protozoa, it must always be borne in mind that in many instances animals recover from certain protozoal infections, such as coccidiosis, not through the development of any immunity, but solely through the normal life cycle changes of the parasite which develop the exogenous phases of the cycle. Once this border line is passed, the organism ceases to be infective to the host except through the original portal of entry. Gradually the schizogonous forms develop the propagative phase, until finally asexual reproduction—the only phase in which auto-infection is known to occur in such forms—has completely given way to sporogony. This, of course, leaves unanswered the question as to how we may account for persistent carriers of coccidial infection. This naturally presupposes either the continuance of the schizogonous cycle as the source of supply of gametocytes, or the reversion of the gametes or gametocytes back to asexual forms. The latter performance is so wholly at variance with established biological principles as to be, to say the least, rather improbable. On the other hand, we are led to wonder what are the conditions that bring about the prolonged series of asexual reproductions while the organism continues to produce oöcysts.



influenced by chemical changes originating in the cells and body fluids of the hosts.

The feeding habits of free-living forms may give a clue to the working out of this problem. They have been extensively studied by many workers, who have brought out important points. Whether the reactions in these phenomena are purely of a chemotactic nature or are combined with changes in surface tension remains to be proved. The significant thing, however, in connection with the theory I have just advanced is that these reactions in the case of parasitic forms are *not restricted to the protozoa, but may be participated in by cells belonging to the host.*

The most striking example of this that I can recall at the present time is that furnished by *Lankesterella* sp. This organism is parasitic in the nucleated erythrocytes of the frog. Neresheimer<sup>\*</sup> has described and figured the penetration of the parasite into the erythrocyte. When the parasite and erythrocyte are separated by a distance about equal to the length of the parasite, amoeboid movements on the part of the erythrocyte become evident, and eventually the blood cell throws out two long, pseudopodialike processes and, as Minchin remarks with characteristic felicity, "opens its arms, as it were, to the parasite, and engulfs it in a manner very similar to the ingestion of food by circumvallation on the part of an amoeba." The parasite is then drawn into the body of the erythrocyte, which rounds out and resumes its normal form with the parasite in its interior. In this instance it must seem that the parasite gives off some substance that, even at a distance, awakens a reaction on the part of the host cell, which brings about its own destruction by aiding the entrance of the parasite. It is conceivable to me that under certain conditions epithelial cells may do the same thing.

The attraction of the gametes of protozoa is a process that is possibly in line with the above and is sufficiently distinct from anything shown by the bacteria to make us believe that in dealing with the factors that determine the relations between protozoan parasites and tissue cells we have something radically different from the conditions that govern bacterial infections.

*Trichomonas* flourishes only in an alkaline medium in the intestine. In the vagina it is found only in mucus having an acid reaction and is quickly got rid of by douching with alkaline

<sup>\*</sup> *Arch. f. Protistenk.* (1909), 16, 187.

fluids." If *Trichomonas intestinalis* is identical with *Trichomonas vaginalis* as some writers, notably Lynch,<sup>(27)</sup> hold, there is one example of adaptation to a changed environment. Lynch, in what is probably the first successful attempt to cultivate *Trichomonas* or experimentally to transmit it to a lower animal, cultivated *Trichomonas* from the vagina and mouth in acidified bouillon and injected a rabbit rectally, with the result that the feces of the rabbit became soft and contained many trichomonads.

Ohira and Noguchi<sup>(40)</sup> on the other hand, cultivated the mouth trichomonad (a tetratrichomonad in their cases) in a slightly alkaline mixture of ascitic fluid and Ringer's solution, which they found much more favorable than acid media. They grew the trichomonads both at room temperature and at 37° C., which was 7 degrees higher than that at which Lynch was able to cultivate the organisms in his medium. Lynch observed no developmental phenomena in his cultures, but Ohira and Noguchi describe multiple fission and the budding off of the daughter cells one by one, as has been described by Kofoid and Swezy<sup>(26)</sup> in the case of *Trichomonas augusta*. Neither Lynch nor Ohira and Noguchi saw cysts. In speaking of *Trichomonas intestinalis*, Rhamy and Metts<sup>(44)</sup> say that the organism lives best in a neutral or slightly alkaline medium "and for this reason any tissue of low vitality will harbor them."

Disregarding for the moment the question as to whether the composition of the intestinal contents at any time influences the division rate of the organism, let us consider the possible factors bearing on the actual entrance of the flagellated organism into the epithelium itself. Several conditions may govern this: 1, the organism may mechanically bore its way in; 2, it may gain entrance through a break in the epithelium brought about mechanically by physicochemical changes or through the action of some other microöganism; or 3, it may secrete some cytolytic agent that attacks the epithelium. The fundamental question is, What stimulus is there that causes the organism to behave in this manner? That is the problem that presented itself to Hadley in his study and that he frankly states cannot be answered with any finality at the present time. In the case of the coccidia and other obligatory cytozoic parasites, it would seem that the organism characteristically and necessarily seeks a home within

<sup>9</sup> Escomel (*Bull. Soc. path. exot.* (1917), 10, 553) states that the reactions of *Trichomonas vaginalis* toward therapeutic agents are the same as those of *Trichomonas intestinalis*. He found that a 1 per 1,000 solution of metallic iodine (freshly prepared) would kill both species instantly. He bases his conclusions on studies in vitro.

the epithelium. But with the flagellates, whose habitat would certainly not seem to be normally in the tissues, the case is different. *Entamoeba*, *Balantidium*, and the flagellates evidently find suitable conditions for life in both the lumen of the intestine and in the tissues and prosper to a greater or lesser extent in either place. If they show a preference, it seems to be in favor of the lumen of the intestine. With the coccidia the performance of tissue invasion is obviously in line with the regular life cycle; with the flagellates and possibly with *Entamoeba* and *Balantidium*, tissue parasitism appears to be in the nature of a departure from the normal.

In *Coccidium*, for example, sporogony seems to fill the dual purpose of protoplasmic rejuvenescence (through fertilization) and the dissemination of spores. It seems to supervene upon sexual maturity and lowered protoplasmic vitality, and yet cases have been reported of chronic coccidiosis, in which the host became virtually a carrier, producing a constant stream of spores of the parasite, which obviously meanwhile continues its schizogonous cycle. Is this a matter of chemical adjustment between host and parasite?

Calkins's classical experiments with *Paramœcium* show the effects of artificial stimulation on the metabolism and reproductive rate of Protozoa and are merely an illustration of the effects that may be produced upon a protozoön by altered environmental conditions. These chemical changes may be, in the future, found to exert a more profound influence on the activities of parasites than we now realize. In 1914 I suggested<sup>(21)</sup> the possibility that relapses of malarial fevers might be brought about by a transient glycæmia, the blood sugar furnishing the rejuvenating stimulation. At the time I had in mind the work of Calkins on *Paramœcium* and that of Bass and Johns on the cultivation of *Plasmodium*. This view has been more recently expressed by Calkins,<sup>(5)</sup> who has suggested that the change may be, as I myself have long held, in the nature of the equivalent of fertilization, a process of the restoration of lost vitality, a substitution of a labile for a stabile condition of the protoplasm. Experimental evidence, as I have stated, points strongly to the belief that such a condition of protoplasmic rejuvenation can be brought about artificially within certain limits.

While we have so far no direct evidence of the invasion of the human tissues by the intestinal flagellates or flagellates of that type, unless we include Ross's observation of "cercomonads" having several flagella and an undulating membrane and tricho-



monads in some cutaneous ulcers, we have considerable evidence that it may occur under certain conditions in the lower animals.

Intestinal parasites representing genera frequently seen in man do, in the lower animals, pass from the lumen of the intestine into the blood and lymph on the development of certain pathological conditions in the host. This fact was early noted by Danilewski in the case of *Hexamitus*, a flagellated parasite in the intestinal tracts of tortoises and frogs. The animals in question had long been in captivity. They showed signs of distress and exhibited œdematous swellings in the muscles and transudation of lymph into the peritoneal cavity. Microscopic examination showed the presence of the protozoa in the blood, lymph, œdemata, and transudations. Plimmer<sup>(41)</sup> has reported a number of similar cases occurring in reptiles and batrachians in the London Zoölogical Gardens. He found both *Hexamitus* and *Trichomonas* in the blood. Plimmer holds that the presence of intestinal parasites in the blood stream is to be associated with definite and recognizable lesions of the intestinal wall. It is fairly well established in connection with the parasitic protozoa that in some cases a parasite, which may be perfectly or relatively harmless when the host is free, may become pathogenic or even lethal when the host is in captivity.

Wenyon<sup>(50)</sup> points out that intestinal flagellates, like the intestinal bacteria, occasionally invade the tissues shortly before or after death. He attributes this to diminished resistance on the part of the intestine, which permits the passage of organisms that normally live in the lumen of the gut. Diminished resistance is a broad term that covers up considerable uncertainty. Is it wholly, in this case, a matter of lowered resistance, are essential chemical changes involved, or do the processes of autodigestion of tissues of the alimentary tract, which may set in soon after death, play their part?

Wenyon quotes Basile, Gonder, and Stevenson on the matter of tissue invasion by the flagellates. Gonder recovered *Lambli*a from the blood stream of a fowl; Basile, *Lambli*a from the liver of a rat. The liver was dotted with white cysts containing the organisms. Basile inoculated a rat peritoneally with the contents of some of these cysts and later found *Lambli*a in the liver and mesenteric glands. Stevenson showed Wenyon sections of the cæcum of a mouse having definite lesions of the mucous surface that had been invaded by numerous trichomonads. *Lambli*a has been shown as invading the glands of the small intestine of the rabbit. Wenyon says:

These are exceptional cases, but so long as they occur there is the pos-



sibility that the invading flagellates will give rise to symptoms of one kind or another. \* \* \* If such invasion can occur [speaking of *Trichomonas*], probably through a surface broken by some other infection, or irritant such as sand, it is possible that the flagellates might aggravate the lesion or produce definite symptoms.

So much for *Lambli*a infections in the lower animals; but Fantham and Porter(12) carry the matter farther in their inquiry into human lambliasis. They say:

We may say at once that in both human and animal lambliasis stools, as well as at post-mortem examinations, erosion and distortion of the intestinal epithelial cells occurred, owing to the direct suctorial action of the flagellate *Lambli*a.<sup>10</sup>

Woodcock(52) holds in the case of flagellate infections in man that if the normal bowel condition becomes disturbed and its resistance lowered the balance between the host and the parasite is no longer maintained, and the parasites will, therefore, multiply more rapidly and in turn bring about a more intense reaction on the part of the host, establishing a vicious circle. He believes that the presence of a vast number of parasites in the intestine "with the concomitant production of waste metabolic substances, is sufficient to irritate the mucosa and give rise to deleterious and toxic effects." He adds:

Fortunately, the flagellates appear to be unable to penetrate the mucosa in the way that *E. histolytica* and *Balantidium* can (given favourable conditions), and therefore, never (?) produce true dysenteric symptoms, the derangement being generally limited to severe or mild diarrhoea.

Minchin(34) raises the old question as to whether the migration of the parasite, when the tissues are involved, is the cause of the diseased state or if the diseased state of the host gives the parasite a chance to spread to other parts of the body. Aside from that point, he makes the exceedingly pertinent remark that the fact that intestinal parasites can pass from the gut to the blood is of phylogenetic as well as of practical importance.

Walker(49) discusses the case of *Balantidium coli* infections in man. He speaks of the absence of liver involvement by this parasite, quoting the old case of Stockvis,(48) in which "*Paramaecium*" (*Balantidium* ?) was recovered from the sputum, the parasites presumably having come from the liver by way of the lung.

In connection with the involvement of the liver in entamœbiasis and balantidiosis it is customary to regard the route of invasion as lying through the portal circulation, the parasites entering the radicles of the portal vein. It must be borne in

<sup>10</sup> The italics are mine.—F. G. H.

mind, however, in connection with the powers shown by *Entamoeba* and *Balantidium* to penetrate deep into the tissues of the intestine that the close apposition of portions of the colon to the surface of the liver make it possible that the liver may become involved as a result of the direct migration of the parasites through the tissues, a contingency that has been in the minds of certain pathologists for some time. The possibility of the conveyance of the parasites through the lymph channels must not be entirely overlooked. The likelihood of retrograde infection through the bile ducts is rather remote, although it is conceivable. Stress has been laid by some of the workers on the presence, more or less frequently, of *Balantidium* in the blood vessels and the lymph spaces, and Bowman<sup>(3)</sup> and Walker<sup>(49)</sup> both report the presence of *Balantidium* in the lymph glands of the mesentery.

Walker states his firm conviction that *Balantidium* will pass through the healthy epithelium. This process, he says, is not accompanied by necrosis or ulceration of the epithelium; it consists of the pushing aside or at the most a rupture of the epithelium. In every case, he says, entrance is through the epithelium and in no case within the tubules. Once within the tissues, the presence of the parasites is certainly associated with necrosis. Walker adds that the lesions and cellular reactions produced in the early stage before being complicated by secondary bacterial invasion are characteristic and are distinguishable from those due to bacterial infection.

In experimental work performed on monkeys, Walker found the epithelium intact "except for mechanical injury due to entrance of the balantidia or to minute hæmorrhages, but no exudate or ulcerations." He also concludes that lesions in the intestinal epithelium from bacterial infection or other causes are not only not necessary for the entrance of the *Balantidium* into the tissues, but that in none of the monkeys in which such lesions existed have the balantidia taken advantage of them to enter the tissues.

Manlove<sup>(28)</sup> cites two cases of balantidial dysentery in man, in support of Walker's contention that *Balantidium* may produce "abscesses" in the intestine that are sterile except for the presence of the protozoön. Walker mentions the fact that the *Balantidium* found in the pig, which he holds to be identical with that infesting man, seldom if ever penetrates the tissue of the pig intestine. On this point I can speak from experience, for I have found *Balantidium* in sections of the pig intestine

not only in the tissue, but in the blood vessels as well; in fact, the microscopic picture was similar to that seen in the case of human balantidiosis.

Walker also points out the fact I have mentioned that an individual may harbor *Balantidium* over long periods of time, apparently without sustaining harm. But sooner or later, he adds, the patient so parasitized develops balantidial dysentery. The periodic appearance of the parasites in the stools that he notes is a phenomenon seen frequently in connection with the intestinal Protozoa as a group.

The oral apparatus of *Balantidium* is similar to that of a number of free-living Heterotrichida, and indeed *Balantidium* is suspected of being capable of existence free in pond water. The oral apparatus of this organism does not seem, at first glance, particularly to adapt it for penetration of the tissues; and if we are to adopt the views of Walker and of Manlove, it remains to discover how this may be brought about.

Bowman quotes Glaessner<sup>(19)</sup> as having recovered a very active diastase and a fairly strong hæmolytic substance in extracts of *Balantidium coli* from the fæces. These ferments exerted no effect on dilute albumen, on peptone solution, or on fibrin. One naturally asks why they should and why they should be concerned in cytolysis. Any such supposition seems to me to be entirely untenable. It seems to be much more probable that Glaessner, after all, merely isolated some of the normal digestive ferments of the parasite. Both diastatic and hæmolytic ferments seem to be exceedingly necessary to the carrying out of the normal anabolic processes in an animal living in such an environment as that in which *Balantidium* lives. A diastatic ferment ("amœbodiastase") has been isolated from amœbæ. Proteolytic ferments have been isolated from other protozoa, and their action has been the subject of considerable study by Nirenstein, Greenwood, Metchnikoff, and others.

It is still an open question whether *Entamœba* and *Balantidium* enter the tissues *through* or *between* the cells and whether the entrance is effected *mechanically* or by the aid of *cytolysins*. Manlove<sup>(28)</sup> quotes Crowell<sup>11</sup> as stating his belief that the question of the essential pathogenicity of *Balantidium* is not yet absolutely settled. As to the invasive power of the organisms, Manlove says:

Doctor Crowell expressed the theory that it seems reasonable to conclude that some substance (toxin?) originating from the balantidia is able to

<sup>11</sup> Crowell, B. C., personal communication.



produce necrosis of the tissues, and the inflammatory phenomena that occur later are the result of the action of the accompanying bacteria. Also one could imagine that this hypothetical toxic substance from the balantidia produces a cytolysis of the epithelial cells of the mucosa with which they come in contact and so forms a portal of entrance to the deeper tissues for the organisms.

Restating this view to me, Crowell<sup>12</sup> holds that it is logical to suppose that, if the balantidia are capable of producing within the tissues a substance that brings about necrosis, this same substance might be employed in the initial penetration of the intact epithelium.

However, it may be pointed out that a fundamental objection that might be opposed to this hypothesis is the fact that substances that are capable of producing marked injury to the unprotected connective and other tissue elements of the sub-mucosa may have no effect whatever on the healthy epithelium, the chief function of which is constantly to protect the underlying tissues from substances that would be injurious. To injure by a toxic substance the intact epithelium would, it seems to me, require a stronger toxin than the changes produced, once having effected penetration, would indicate. That is, of course, provided there is no specific epitheliolysin, of which there is no evidence. On the other hand, much of the evidence I have already quoted points to the *mechanical* invasion of the intestine, rather than to the employment of cytolysins. Walker has stated his belief that entrance is accomplished by the pushing aside or rupture of the epithelium, and similar evidence in the case of *Trypanosoma lewisi* seems to have been produced by Minchin and Thomson<sup>(36)</sup> and by Nöller,<sup>(39)</sup> in the case of *Trichomonas* by Hadley, and in the case of *Trypanosoma gambiense* by Hindle.<sup>(22)</sup>

Carrying the case to the parasitic Rhizopoda, the active, powerful pseudopodia, formed mainly of ectoplasm as seen in *Entamoeba histolytica*, are in marked contrast to the relatively blunt, inactive pseudopodia of *Entamoeba coli*, which contain comparatively little ectoplasm, and suggests the possibility that it is this difference in the structure of the pseudopodia that enables *Entamoeba histolytica* to push its way between the cells. Are cytolytic agents necessary here? Or, in the case of *Balantidium*, are the cell membranes or bridges between the cells able to withstand the powerful currents set up by the cilia

<sup>12</sup> Crowell, B. C., personal communication.



and membranelles of the adoral zone of the organism when closely and persistently applied to the surface of the tissue? Once the entrance is forced, it would be about as easy for one of these ciliates to worm its way in as it would be for an amœba. Their bodies are plastic to a degree that at times superficially resembles pseudopodia formation, and they accommodate themselves to every obstacle they meet as may be readily seen by any one who will take the time to study a cover slip preparation from a case of *Balantidium* infection containing actively moving parasites.

After having gained entrance to the tissues, it seems to me for the present reasonable to believe that the nutrition of these organisms continues to be holozoic within the tissues, so we are left to speculate as to whether necrosis in the tissues, where involvement is unaccompanied by the presence of bacteria, is due to the secretion by the organism of some special cytolytic ferment, or whether it is brought about by the action on the tissues of the normal katabolic products of the parasites, a theory which seems to me not wholly devoid of reason. This may also apply as well to *Entamœba histolytica* as to the flagellates and ciliates.

But aside from tissue penetration, the situation contains further inconsistencies. *Balantidium*, from such reports and personal experiences as are available to me at this time, seems to be more frequently seen in the blood vessels of the intestine than *Entamœba*. In spite of this, however, *Balantidium* is never found in the liver; whereas *Entamœba* is very frequently found there and in other parts of the body. Why is this?

May we not find our answer in the general tendency of the flagellated and ciliated protozoa, spermatozoa, and the like to swim *against* the current, whereas the relatively passive entamœbæ are carried willy-nilly by any current to a point where they meet with some obstruction, finally to lodge and reproduce, provided the conditions are favorable? *Balantidium* and *Trichomonas*, with their powerful, well-developed motile organs, would surely have less trouble in stemming the tide of the slow-moving venous and lymph circulation than even the poorly equipped spermatozoön has in breasting the current of the ciliated Fallopian tubes. And yet the population of the world attests the success of the spermatozoön, and the absence or comparative absence of extra-intestinal infections with *Balantidium*, *Trichomonas*, *Lambliæ*, and the like seems to betoken equal

success on the part of these organisms in avoiding being carried away. This apparently constitutes an application of the principle of the "avoiding reaction" as described by Jennings<sup>(24)</sup> in his studies on the tropisms in the Protozoa. In the smaller vessels of the intestine, aside from the obstruction afforded by the walls of vessels having a caliber smaller than the diameter of the parasite, the flow is probably too slow to bring about the degree of stimulation necessary to excite this reaction. The parasites would probably be prevented by the excessive blood pressure from even entering the arteries, but granting that they might do this, the stimulation would probably pass above the *optimum*, and the organisms would be passively swept back.

But in the larger radicles, where the current is *away* from the intestines, it is conceivable that the flow would be of about the right intensity to cause the parasites to about face and turn "upstream." At the same time it would be strong enough to sweep the entamœbæ toward the liver. If this hypothesis will hold, it would be added evidence to the effect that in hepatic involvement the main path would be through the portal circulation. Lastly, however, it must not be forgotten that there still remains the possibility that the balantidia may reach the liver, but find the conditions for continuation of life unsuitable at that site. If Stockvis's observation is correct, that contingency seems to be ruled out. The possibility of the conveyance of the cysts of *Balantidium* to the liver through the blood stream appears to be too remote for consideration at this time. There is no definite proof that *Balantidium* forms cysts in the tissue, and it would require to be shown that the cysts would open under the influence of any agent that is likely to be present in the liver. Normally these cysts may be expected to open under the influence of some digestive ferment only, though it is well known to protozoölogists that certain protozoan cysts will open under the influence of ferments secreted by bacteria or through external or internal influences of an uncertain nature. The cysts of the intestinal parasites, however, appear to be adapted to the action of the intestinal juices.

Turning back to the flagellates, let us review what evidence we actually have of their tissue-invading powers.

Minchin and Thomson, in their great memoir on *Trypanosoma lewisi* and its relations to the rat flea *Ceratophyllus fasciatus*,<sup>(36)</sup> describe in considerable detail an intracellular stage in the stomach of *Ceratophyllus* following the penetration of the epithelium. They were not so fortunate as to observe the actual

penetration of the cell by the parasite, but Nöller, (39) who did, states that he saw—

a trypanosome of which the pointed hinder end had already penetrated into an epithelial cell. The flagellum-bearing anterior end beat violently and incessantly, whereby the trypanosome penetrated further and further into the cell. After I had watched this spectacle for about five minutes the trypanosome, which had so far penetrated into the cell as far as the middle of its body, suddenly shot into the cell and stirred up the granular cell contents by its lively movements. Since, however, the cell was torn on its opposite side the trypanosome shot out of the cell again.

In discussing the type of cell attacked by the trypanosomes, Minchin and Thomson are somewhat in agreement with the observations of Walker. They never saw intracellular stages of the trypanosomes in the cells of the epithelial crypts. The trypanosomes never occurred in any cell that was not definitely a part of the general epithelium, and the appearances they saw seemed to suggest that "the attack is usually made on the side of the cell; the occasional, though rare occurrence, however, of intracellular stages in quite young cells, shows that the trypanosomes can penetrate into epithelial cells before the separation between them has developed."

Of course, the general character of the epithelium of the alimentary tract of the flea probably differs in important details from that of the epithelium in the human digestive tract, but the conditions that exist in certain cells and that may profoundly influence tissue invasion are suggested in the following paragraph from the paper of Minchin and Thomson:

The trypanosomes attack by preference the fully-formed, but still young and vigorous cells, which may contain granules of the normal type and even yellow bodies, but no fatty deposits; cells which may be well characterized as adolescent in type, and which, stain a clear, light-grey with iron-hæmatoxylin after Flemming-fixation. It is in such cells that the earlier stages of the intracellular multiplication are to be found in flourishing condition and often in considerable numbers; but if the trypanosomes are numerous the cell soon becomes exhausted.

Evidence of the possibility of the transmission in man of *Trypanosoma gambiense* by coitus was brought forward by Koch, (25) in 1907, and the transmission of *Trypanosoma equiperdum* in dourine has long been an established fact, although trypanosomes have not been demonstrated in the seminal fluid, and the possibility of even slight abrasions of the mucous membranes of the contracting parties still remains.

Hindle (22) has made a study to determine the possibility of the entrance of trypanosomes through the intact skin and mucous



membranes. Using *Trypanosoma gambiense*, he was able to secure infections per os, care being taken to prevent the possibility of producing lesions in the mouths of the rats used. Attempts to secure infection by coitus between rats were unsuccessful, but careful experiments in infection through the vagina were successful. Infection was also secured by applying the infected blood to the unshaved skin of rats, and Hindle concludes that *Trypanosoma gambiense* is able to penetrate the sound mucous membrane and the undamaged skin.

It seems from this that certain, at least, of the flagellates experience no difficulty whatever in penetrating cells and tissues. Minchin and Thomson have shown that apparently the side of the cell is the most vulnerable point. Furthermore they cite the adhesive properties of the flagellum under certain circumstances when the organism may attach itself to the epithelial surface by its flagellum and then bore its way in, posterior end first. Might not a similar action be possible in the case of *Trichomonas*, where the axostyle would play an important part? This, of course, apart from the condition where *Trichomonas* might assume an amœboid form, as described by Castellani(7) for *Entamœba undulans*, and bore its way in by its long, powerful pseudopodium. *Crithidia* is known to attach itself to epithelium by its shortened flagellum. This would give us, then, three separate relations existing between intestinal flagellates and the epithelium:

1. Close application to the surface of the epithelium without actual penetration. *Lamblia*.

2. Attachment by a flagellum, which may penetrate the cell membrane. *Crithidia*.

3. Actual penetration of the cell and entrance of the entire organism into the cell body and subsequent liquefaction of the cell contents. *Trypanosoma*, *Trichomonas*, *Hexamitus*, and possibly *Lamblia*.

The amœboid, flagellated, and ciliated protozoa of the alimentary tract represent a group standing apart from the other protozoan parasites. For the most part they are, in regard to structure, mode of life, and nutrition, not very different from free-living forms we may find in almost any mud puddle or watering trough. That they show certain tendencies toward development to obligatory tissue parasitism cannot be denied, but they can scarcely be said to have "arrived."

The amœboid forms as represented by the entamœbæ are strikingly similar in structure and mode of life to *Vahlkampfia*



and other small free-living amœbæ, while *Chlamydomphrys stereocorea* closely resembles some of the free-living testate forms.

The flagellates exhibit elaborate motile apparatus and, in many cases, well-developed mouth parts for the ingestion of food.

The ciliated parasites such as *Balantidium*, *Nyctotherus*, *Diplodinium*, *Ophryoscolex*, and the like not only resemble free-living forms in regard to their motile and food-getting organs, but many of them are highly organized in other ways, through the possession of neuromotor apparatus. *Opalina* seems to constitute an exception to the rule.

The absence in most parasitic forms of the contractile or excretory vacuole, which is cited by many as a characteristic of the parasitic protozoa, is not by any means necessarily an adaptation purely to a parasitic mode of life. It is an adaptation, to be sure, but parasitism is not the only thing that determines it. The marine species of protozoa for the most part do not possess contractile vacuoles. The gradual transfer of a marine species to a fresh-water environment will frequently cause it to develop a contractile vacuole, which it will lose on its return to salt water. So that the possession or absence of this organelle seems to be governed largely by the osmotic tension of the medium in which the organism finds itself.

In other words, Are these forms to be considered as on the same plane with the blood and obligatory tissue parasites as the trypanosomes and coccidia? It would seem not. The intestinal flagellated and ciliated protozoa of man, with the exception of *Lambliæ*, do not show evidences either of a high degree of adaptation to a parasitic mode of life or evidences of structural degeneration as a result of parasitism. They have not found themselves. They are, in a measure, creatures of impulse subject to the play of natural forces that are yet to be understood, responding to frequently changing stimuli by varied reactions that are the despair of the physician and parasitologist alike. They have yet to settle down and behave with the almost mathematical regularity that we have grown to expect of their more conventional brethren, such as the coccidia and trypanosomes.

With the flagellates it appears that tissue parasitism is a departure from the normal and with only lesser force would this seem to apply to *Balantidium* and *Entamœba*. It is perfectly clear that many of these parasites may live in the intestinal tract over long periods of time without causing trouble—perhaps they may never cause trouble during the life of the host. On the other hand, the day may come when some con-

dition arises—lack of resistance on the part of the host or some other change in the host that intervenes to endow the parasite with new powers, as has been hinted by Hadley—and the parasite strikes boldly out into a new field, the tissues. What these conditions are remains to be determined. That they can be explained on the same ground as we explain bacterial invasions has been a popular supposition, which it seems to me would be wise for us to abandon for the present at least and to look for something new. We now know considerable about the reactions between *animals* and *plants* (bacteria), and it seems about time we added to our knowledge regarding the reactions between host *animals* and parasitic *animals*.

Other points of inquiry bear on the discovery of the conditions under which a parasite is harmless to its host when the latter lives free but becomes pathogenic or even lethal when the host is in captivity and of the possible free life of certain intestinal protozoa as suggested by Rhamy and Metts, Escomel and Smithies, and supported by laboratory experimentation.

The time has arrived when workers in the field of parasitology should fairly face the situation presented by the intestinal flagellates. They have been under suspicion for many years, during which time there has been gradually accumulating a mass of evidence against them. No progress will be made if we are to continue to employ, as our criteria of pathogenicity or non-pathogenicity, the presence or absence of blood and pus in the stools. Among the possible effects that may be produced by these organisms may be mentioned:

1. The production of antigrowth vitamins or growth-inhibiting substances, as suggested by Gibson.
2. The production of substances directly toxic.
3. Unfavorable effects upon the host through the liberation of the products of metabolism of the parasite.
4. Mechanical irritation of mucous surfaces by the parasites when present in large numbers.
5. Interference with absorption in the intestine through the adherence of large numbers of parasites to the surface of the epithelium, as in the case of *Lamblia*.
6. Actual invasion and destruction of the tissues with all its concomitants and sequelæ.

Analysis of the work of Castellani on *Entamæba undulans*, of Gauducheau on *Entamæba* and *Trichomonas*, and of Hadley and others on the tissue-invasive power of *Trichomonas* and the

other intestinal flagellates seems to be suggestive of what may be expected in man in regard to these parasites.

Next in order appears to be the desirability of attempting to explain the conflicting opinions expressed by different authors regarding the pathogenicity of the intestinal flagellates. It has been shown that some workers regard these forms as harmless or capable, at the most, of giving rise to nothing worse than diarrhœa, while others frankly express the belief that they may produce dysentery—actual lesions of the bowel. Is it possible that we have here different strains of the same organism, some showing and others not showing tissue-invasive powers—a condition somewhat resembling the relation between *Entamœba histolytica* and *Entamœba coli*?

Another problem is that of cross-infectivity. Many of the genera found in lower animals are found in man. Are the species found in the lower animals capable of life in man? The rule is known to apply in the case of *Balantidium*, it seems to be the case with *Lambliæ*, and Lynch has produced evidence to show that the rat is a true host and not merely a carrier of *Entamœba histolytica*.

The actual invasion of the tissues of the human intestine by the flagellated parasites remains to be demonstrated, but it may have occurred and we may have passed it by. It seems conceivable, as I have suggested, that *Trichomonas* in the tissues may so closely resemble *Entamœba* as to have been mistaken for the latter. Evidence that tissue invasion by these same parasites occurs in the lower animals seems to be sufficiently convincing.

However, it is still to be proved how this takes place. To my mind the evidence, so far at least as the flagellates and ciliates are concerned, seems to favor mechanical penetration of the tissues rather than entrance with the aid of cytolytic agents. But it is also a fact that necrotic changes in the tissues may be associated with the presence there of the parasites. These changes might be due either to definite cell-destroying ferments, or might simply be the result of the action of katabolic products of the parasites that happen to be toxic to the cell elements. From the evidence, cytolytic ferments appear to be unnecessary in many cases, certainly with many of the flagellates and possibly in the cases of the ciliates and amœboid forms.

But this should not preclude a study of the cytolytic enzymes that may be produced by these organisms. If they exist, the investigations should include an inquiry into the conditions under which they are formed with a view to discover whether they are constantly secreted or are produced only under special in-



fluences either existing at some definite period of the life cycle of the parasite or originating in the host.

Enough has been shown effectually to despoil *Trichomonas* and its cousins of the reputation for harmlessness to man they have previously had. It remains to determine some effective remedy for them.

With this should be coupled a closer study of the bionomics of the protozoa parasitic in the alimentary tract. It is only through such a study that we can hope to secure the knowledge of the intricate and often seemingly anomalous relations between these parasites and their hosts that will lead us to a better understanding of their activities and that will make it possible to discover the appropriate means for controlling the conditions to which they give rise, from the viewpoints of both cure and prevention.

#### ADDENDUM

Since the foregoing was written, several papers dealing with various phases of the problem of flagellate infection of the intestine have come to hand. To deal with them would involve the resetting of a considerable portion of this paper. Particularly worthy of the attention of those who are interested in the subject is the paper of Chalmers and Pekkola,<sup>13</sup> in which they discuss *Chilomastix mesnili* and give a systematic review of the Tetramitidæ. Gäbel in his paper on the pathogenic flagellates<sup>14</sup> gives an extensive bibliography of the literature on flagellate diarrhœa.

#### REFERENCES

- (1) ALEXEIEFF, A. Kystes de *Trichomonas intestinalis*. *Compt. rend. Soc. biol.* (1911), 71, 296.
- (2) BARLOW, NATHAN. Studies on *Trichomonas*. *New Orl. Med. & Surg. Journ.* (1916) 69, 299.
- (3) BOWMAN, FRED B. Two cases of *Balantidium coli* infection with autopsy. *Phil. Journ. Sci., Sec. B* (1909), 4, 417.
- (4) BRUMPT, E. Cõlite a *Tetramitus mesnili* (Wenyon 1910) et cõlite a *Trichomonas intestinalis* Leuckart 1879. *Blastocystis hominis* n. sp. et formes voisines. *Bull. Soc. path. exot.* (1912), 5, 725.
- (5) CALKINS, G. N. General biology of the protozoan life cycle. *Am. Nat.* (1916), 50, 257.
- (6) CASTELLANI, ALDO. Notes on tropical diseases met in the Balcanic and Adriatic zones. *Journ. Trop. Med. & Hyg.* (1917), 20, 198.
- (7) IDEM. Observations on some protozoa found in human fæces. *Centralbl. f. Bakt., Orig.* (1905), 38, 67.
- (8) CHATTERJEE, G. C. Note on flagellate dysentery. *Ind. Journ. Med. Res.* (1917), 4, 393.

<sup>13</sup> *Ann. Trop. Med. & Parasit.* (1918), 11, 213.

<sup>14</sup> *Arch. f. Protistenk.* (1914), 34, 1.

- (9) CHATTON, EDUARD. Les "Blastocystis" stades du cycle évolutif de flagellés intestinaux. *Compt. rend. Soc. bñ.* (1917), **80**, 555.
- (10) DERRIEU et REYNAUD. Dysenterie chronique à flagelle nouveau. *Bull. Soc. path. exot.* (1914), **7**, 571.
- (11) ESCOMEL, E. Sur la dysenterie à Trichomonas à Arequipa (Perou). *Bull. Soc. path. exot.* (1913), **6**, 120.
- (12) FANTHAM, H. B., and PORTER, ANNIE. The pathogenicity of Giardia (Lambli) intestinalis to men and to experimental animals. *Brit. Med. Journ.* (1916), 2900, 141.
- (13) FANTHAM, H. B., STEPHENS, J. W. W., and THEOBALD, F. V. The Animal Parasites of Man. John Bale, Sons & Danielsson, Ltd., London (1916), 56.
- (14) IDEM. Ibid., 59.
- (15) GALLI-VALERIO, B. Note de parasitologie. *Centralbl. f. Bakt., 1. Abt., Orig.* (1900), **26**, 305.
- (16) GAUDUCHEAU, A. Recherches sur multiplication des entamibes. Trans. 2d Biennial Med. Congr. Hong Kong. Noronha and Co., Hong Kong (1912), 74.
- (17) GIBSON, R. B. Recent nutritional investigations on maintenance and growth. *Proc. Phil. Vet. Med. Assoc.* (in press). Manila, Feb. 5 to 9, 1918.
- (18) GIBSON, R. B., and CONCEPCION, ISABELO. The influence of fresh and autoclaved cow's milk on the development of neuritis in animals. *Phil. Journ. Sci., Sec. B* (1916), **11**, 119.
- (19) GLAESSNER, K. Ueber Balantidienenteritis. *Centralbl. f. Bakt. Abt. Orig.* (1908), **47**; 351.
- (20) HADLEY, PHILIP. The case of Trichomonas. *Am. Nat.* (1917), **51**, 208.
- (21) HAUGHWOUT, F. G. See discussion on paper of C. C. Bass. *Am. Journ. Trop. Dis. & Prev. Med.* (1914), **2**, 204.
- (22) HINDLE, EDWARD. The passage of Trypanosoma gambiense through mucous membranes and skin. *Parasit.* (1911), **4**, 24.
- (23) IJIMA, J. On a new rhizopod parasite of man. *Ann. Zoöl. Japon.* (1898), **2**, 85.
- (24) JENNINGS, H. S. The Behavior of the Lower Organisms. Columbia University Press, New York (1906), 73.
- (25) KOCH, R. Cited by Hindle (No. 22).
- (26) KOFOID, C. A., and SWEZY, OLIVE. Mitosis and multiple fission in trichomonad flagellates. *Proc. Am. Acad. Arts & Sci.* (1915), **51**, 289.
- (27) LYNCH, K. M. Trichomoniasis of the vagina and the mouth. *Am. Journ. Trop. Dis. & Prev. Med.* (1915), **2**, 627.
- (28) MANLOVE, C. H. Two cases of balantidial colitis. *Phil. Journ. Sci., Sec. B* (1917), **12**, 149.
- (29) MARTIN, C. H., and ROBERTSON, M. Further observations on the cecal parasites of fowls, with some reference to the rectal fauna of other vertebrates. Part I. *Quart. Journ. Microscop. Sci.* (1911), **57**, 53.
- (30) MATHIS, C. Troubles intestinaux dus au protozoare flagellé, Lambli intestinalis (Lambl 1859). *Bull. Soc. Med. Chirurg. Indo-chine* (1914), **5**, 315.
- (31) MELLO-LEITAO. Cited by Fantham, Stephens, and Theobald, p. 624 (No. 13).

- (32) MILLER, W. W. Hepatozoön perniciosum (n. g., n. sp.), a haemogregarine pathogenic for white rats; with a description of the sexual cycle in the intermediate host, a mite (*Lelaps echidninus*). *Bull. Hyg. Lab., U. S. Pub. Health & Marine Hosp. Service* (1908), No. 46.
- (33) MINCHIN, E. A. An Introduction to the Study of the Protozoa. Edward Arnold, London (1912), 17.
- (34) IDEM. *Ibid.*, 258.
- (35) IDEM. *Ibid.*, 271.
- (36) MINCHIN, E. A., and THOMPSON, J. D. The rat trypanosome, *Trypanosoma lewisi*, in its relation to the rat flea, *Ceratophyllus fasciatus*. *Quart. Journ. Microscop. Sci.* (1914), 60, pt. 4, n. s. 468.
- (37) MIURA, K. Amöbenbefund in der Punctionsflüssigkeit, bei Tumoren der Peritonealhöhle. *Mitt. a. d. med. Fakult. d. kais. Univ. z. Tokio* (1901), 5, 1.
- (38) MUSGRAVE, W. E. Amœbiasis: its association with other diseases, its complications, and its after effects. *Phil. Journ. Sci.* (1906), 1, 547.
- (39) NÖLLER, W. Die Übertragungsweise der Rattentrypanosomen durch Flöhe. *Arch. f. Protistenk.* (1912), 25, 386.
- (40) OHIRA, TOKUZO, and NOGUCHI, HIDEYO. The cultivation of *Trichomonas* of the human mouth (*Tetratrichomonas hominis*). *Journ. Exp. Med.* (1917), 25, 341.
- (41) PLIMMER, H. G. A report on deaths at the Zoölogical Gardens during 1908. *Proc. Zoöl. Soc., London* (1909), 125.
- (42) PRENTISS, ELLIOTT C. Observations upon certain protozoal infections of the digestive system. *New Mexico Med. Journ.* (1915), 208.
- (43) PROWAZEK S. VON. Einige parasitische Flagellaten. *Arbeit. a. d. Kais. Gesundh.* (1904), 21, 1.
- (44) RHAMY, B. W., and METTS, F. A. Flagellate protozoa as an etiologic factor of dysenteric diarrhœa. *Journ. Am. Med. Assoc.* (1916), 66, 1190.
- (45) SCHAUDINN, F. Cited by Wenyon (No. 51).
- (46) SMITH, THEOBALD. Aberrant intestinal protozoan parasites in the turkey. *Journ. Exp. Med.* (1916), 23, 293.
- (47) SMITHIES, FRANK. The occurrence of *Trichomonas hominis* in gastric contents with a report of two cases. *Am. Journ. Med. Sci.* (1912), 144, 82.
- (48) STOCKVIS, B. J. *Paramaecium* in sputa. *Weekblad van h. Nederl. Tijdschrift voor Genesk.* (1884). Nov. 20, 4.
- (49) WALKER, E. L. Experimental balantidiasis. *Phil. Journ. Sci., Sec. B* (1913), 8, 333.
- (50) WENYON, C. M. The common intestinal protozoa of man. *Lancet* (1915), 1173.
- (51) IDEM. Observations on the intestinal protozoa of mice. *Arch. f. Protistenk.* (1907), 1, suppl., 169.
- (52) WOODCOCK, H. M. Protozoölogical experiences during the summer and autumn of 1916. *Journ. Roy. Army Med. Corps* (1917), 29, 290.
- (53) WOODRUFF, L. L. The effects of culture medium contaminated with the excretion products of *Paramecium* on its rate of reproduction. *Proc. Soc. Exp. Biol. & Med.* (1911), 8, 100.



## ILLUSTRATION

**FIG. 1.** Growth curves for dogs fed on fresh and autoclaved milk.

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## PRELIMINARY REPORT ON VARIOUS METHODS OF SERUM APPLICATION IN BACILLARY DYSENTERY<sup>1</sup>

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### TWO TEXT FIGURES

Since the announcement to the world of the specific therapy of bacillary dysentery introduced by Shiga in 1898, this form of treatment has fallen into a state of apparent lethargy in the hands of the medical profession probably because of the failure of other observers to secure confirmatory results. It is only during recent years that the "renaissance," so to speak, of the biological applications of this treatment has attracted many followers. Leading observers who have contributed much to the knowledge of this subject are Shiga, Kruse, Rosenthal, Kraus and Doerr, Rosculet, Vaillard and Dopter, Irimescu, Karlinski and Ludke.(2)

The specific treatment is based on purely biological principles. By immunizing animals, Shiga, the pioneer in this field, could produce sera with which he was able to decrease the mortality rate of endemic dysentery in Japan from 35 to 9 per cent.(4) During an epidemic in 1905 in Rumania Rosculet used prophylactic injection of serum in 18 persons, while 18 other persons exposed under the same conditions were used as controls. While none of the exposed persons who received serum showed any symptoms of the disease, the controls developed bacillary dysentery. Bahr has claimed that polyvalent sera are both antitoxic and bactericidal.(1) These facts themselves speak highly for the specific treatment of the disease and its bright future in connection with the treatment of the disease.

Other investigators, however, have pronounced the serum treatment of bacillary dysentery a failure. It is probable that (a) the serum at their disposal was defective, (b) the treatment was begun too late, or (c) the diagnosis had not been confirmed bacteriologically and the etiology of the disease was not established. Under such circumstances, what beneficial effects could

<sup>1</sup> Read before the Manila Medical Society, December 3, 1917. Received for publication January 17, 1918. The serum used in this work was supplied by the Bureau of Science.



be expected from the administration of antidysenteric serum? On the other hand, experiences of those observers who have made careful studies of serum therapy as applied to this disease speak in favor of this method of treatment.

According to the present communication the use of specific treatment seems to be an effective means of checking the progress of the disease in cases of true bacillary dysentery. Out of 20 cases that I have so far had the opportunity of treating, only one has died. Different methods of administering the serum, namely, intramuscularly, intravenously, and per rectum (serum enema), have been employed.

During September, October, and November, 1917, there were admitted to the medical wards of the Philippine General Hospital 20 cases of bacillary dysentery, which were treated with serum. Diagnosis in all these cases was based upon clinical symptoms and laboratory examinations. Out of 17 cases in which cultures were made from the fæces, six were negative. Of the 11 cases that were found to be positive, two were of the Shiga and one of the Flexner type. Further differentiation of the rest was not made owing to the fact that there were no media available for this purpose.

Of these 20 cases, five were treated medicinally combined with intramuscular injection of serum with one death; six cases intramuscularly with no deaths; three cases treated both with intramuscular injections and antidysenteric serum per rectum with no mortality; three cases treated solely with serum per rectum with no deaths; and finally three cases treated intravenously, with no deaths (see tables).

TABLE I.—*Drugs and serum intramuscularly.*<sup>a</sup>

Case.	Name.	Age.	Sex.	Result.	Culture.	Complication.	Remarks.
1	C. R.	26	F	Recovered	-----	-----	
2	R. C.	18	M	do	-----	-----	
3	E. G.	17	M	do	No media.	-----	
4	J. G.	25	F	do	-----	-----	
5	J. D.	29	F	Died	-----	Lobar pneumonia.	Patient had been sick sixteen days and was in collapsed condition on admission. No autopsy performed.

<sup>a</sup> In the culture column in this and following tables — means dysentery bacilli were not obtained in cultures, though sought for; + means dysentery bacilli were obtained in cultures. When the type of dysentery bacilli isolated was identified, it is so indicated (see text). In each case, search was made for amœbæ.

TABLE II.—*Serum intramuscularly alone.*

Case.	Name.	Age.	Sex.	Result.	Culture.
6	G. S.	22	F	Recovered .....	
7	E. R.	22	F	do .....	
8	C. E.	42	M	do .....	
9	F. B.	22	F	do .....	No media.
10	A. L.	21	F	do .....	Do.
11	L.	26	M	do .....	

TABLE III.—*Serum intramuscularly and per rectum.*

Case.	Name.	Age.	Sex.	Result.	Culture.
12	E. C.	19	M	Recovered .....	
13	E. A.	27	M	do .....	+ (Shiga)
14	J. M.	24	M	do .....	+

TABLE IV.—*Serum per rectum alone.*

Case.	Name.	Age.	Sex.	Result.	Culture.
15	S. T.	45	M	Recovered .....	
16	G. R.	14	F	do .....	+ (Flexner)
17	G. R.	16	M	do .....	

TABLE V.—*Serum intravenously alone.*

Case.	Name.	Age.	Sex.	Result.	Culture.	Remarks.
18	E. B.	20	F	Recovered .....	Shiga +	1 day before treatment.
19	A. M.	30	M	do .....	—	10 days before treatment.
20	Z. C.	37	M	do .....	—	Do.

Table I is intended to show the cases in which drug therapy was combined with serum treatment. With the exception of a single case that died, having been admitted to the hospital in a condition of collapse at the end of sixteen days of sickness, all the other cases were of moderate severity. The average duration of treatment, excluding one case that succumbed, was four and one-half days.

Table II shows the cases treated intramuscularly with serum alone. In this group one case (8) was severe, the rest were only moderately so. The average duration of treatment was four days.

Table III presents cases treated with serum intramuscularly and per rectum. These were all severe, a fact which very likely

is responsible for a longer average duration of the disease, namely, seven days. Case 12, for instance, was having almost continuous bowel movements consisting of a few drops of pure blood every few minutes. The patient was able to count only large evacuations; he gave thirty-five as the number of evacuations during twenty-four hours, which is probably five times less than the actual number of bowel movements.

Table IV presents cases treated with serum per rectum only. All these cases were moderately severe. The average duration of treatment was four days.

Table V presents cases treated intravenously with serum. These cases were all severe. Patients were able to count only large evacuations, for they were passing stools consisting wholly of blood almost continuously. The average duration of treatment was five days.

The serum per rectum was given in the following way: The patient is in the knee-chest position. The injection of the serum was preceded by a cleansing enema of 1.5 per cent solution of sodium bicarbonate; this was followed by another enema of starch solution with a few drops of tincture of opium (60 cubic centimeters with 10 drops of tincture of opium) to diminish the irritability to the intestine; a half hour later the serum was given per rectum. The amount of serum used was from 30 to 50 cubic centimeters daily, depending upon the severity of the case, although the serum can be frequently given without any danger and in larger doses.

The intramuscular administration of serum was done with the usual aseptic precautions. Twenty cubic centimeters of the serum were given twice a day, usually injected into the buttock. Larger doses may be given, depending, of course, on the severity of the case. Willmore advises the injection of 80 to 120 cubic centimeters daily in desperate cases;<sup>(7)</sup> Lukis administers 20 cubic centimeters four times a day every six hours.<sup>(3)</sup> Bahr emphasized the necessity of first cleansing the bowel by the use of a saline purgative, preferably sodium sulphate, so as to accelerate the repair of the ulcerated mucous membrane and also to eliminate unabsorbed toxins, which are responsible for the symptoms of collapse frequently met with in acute and severe cases.

Last of all is the use of serum by intravenous injection. It was done by the closed method and under rigid asepsis. Usually the median basilic vein was selected, being commonly prominent. To avoid anaphylactic symptoms, one cubic centimeter of the



serum may be previously injected intravenously about six hours before the full dose is given. My dosage was 10 cubic centimeters every other day, although Sandwith has given 20 cubic centimeters (of the Lister Institute preparation) daily to adults and 10 cubic centimeters to children.<sup>(6)</sup>

It is not the purpose of this paper to discuss the clinical symptoms of the disease, for these are generally well known. The main point that I wish to bring out is the broader application in the field of medicine of a therapy based on scientific principles.

Again I have to state that it has long been definitely known that specific bacteria are the causative factors of this disease and that they produce definite pathological changes, particularly in the large intestine. Observations of those writers who have made careful studies of this disease have led to the conclusion that the toxins act locally and, when absorbed in large amount, produce toxæmia. Experiments on animals support these views. Injection of dysenteric toxins into susceptible animals produces similar symptoms and anatomical changes in the large intestine to those observed in human beings. These are the findings of Flexner and Sweet and of Doerr.<sup>(2)</sup> Doerr, however, could save the animals from the effects of a lethal dose of toxin by previous injections of serum. Similar experiments were reported by Todd and later by Vaillard and Dopter.<sup>(2)</sup> Sandwith states that serum is both antitoxic and bactericidal.

In view of the established facts set forth by authentic observers on this subject, the local use of the serum seems, to my mind, not to be unscientific. Considering well the morbid changes in the large intestine, where acute inflammation and ulcerations are taking place, and recalling that it is the site where the virus flourishes the best, continuously elaborating the toxins, we can see at once the working basis of the local application. As it has been found that the serum is both antitoxic and bactericidal, it would mean, then, the neutralization, locally, of the unabsorbed toxins and the decreased vitality of the virus, if not its actual death.

As to how far these aims are accomplished, I am not yet in a position to state; but it suffices to say that twenty-four hours after the administration of serum per rectum the patient feels a marked alleviation of the local symptoms. The colicky pain is markedly diminished; the stools may still be very bloody, yet greatly decreased in number; and the temperature is lower. During successive days the stools gradually become less bloody



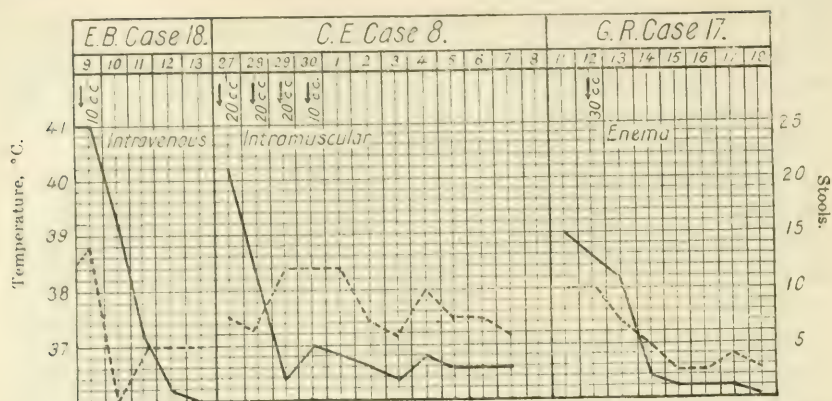


FIG. 1. Cases 8, 17, and 18. Solid line, stools; broken line, temperature. One square is equivalent to one stool.

and mucoid, then feculent, then of a soft consistence, finally becoming normal. I am fully aware of the drawbacks of this method. The serum may not reach the whole area of tissue involved, or it may fail to neutralize the toxins already absorbed in the system, as has been observed in cases 13 and 17. In these two cases charts were made, showing the comparative effects of the serum given per rectum both on the temperature and stools. It is an illustration of a case where serum per rectum is a failure, because the toxin has been already absorbed by the system.

The advisability of the administration of serum by intramuscular injection can be readily seen. Knowing that the toxins of the microorganisms have been already absorbed, naturally we expect the neutralization of the poisons by the serum, which contains specific antibodies. The results are gratifying. If

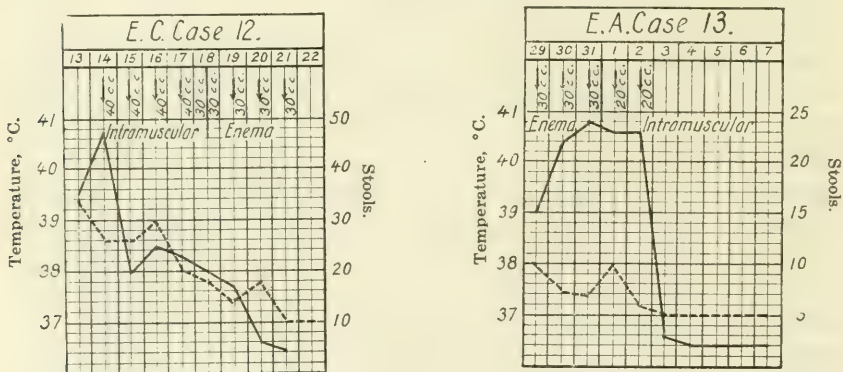


FIG. 2. Cases 12 and 13. Solid line, stools; broken line temperature. One square is equivalent to one stool.

the serum is given, the patient, formerly restless, becomes quiet, the temperature gradually falls, the colicky pains and tenesmus are less severe, and the stools are diminished in number. These results may be observed after from twenty-four to forty-eight hours. In case of collapse, which is present frequently in severe cases, the pulse becomes fuller and stronger a few hours after injection. There is marked abatement of subjective symptoms on the following days. In my experience it seems that the combination of intramuscular administration and serum enemata in acute cases is advisable, for we combat the disease from two sides, namely, (a) neutralization of the toxins in the blood and (b) direct action of the serum on the bacteria and their poisonous products in the lower part of the large intestine. The response of the different patients to administration of serum is shown in fig. 1 (case 8) and in fig. 2 (cases 12 and 13).

The action of the serum introduced intravenously is similar to that of the intramuscular administration, but the effects are more rapid, as shown in fig. 1 (case 18). In the three cases that I treated intravenously with serum, one case was of one day's duration before treatment, and the results are seen in fig. 1 (case 18). In the other two cases, in which the duration of the disease was ten days before treatment, the effect of the serum was not so rapid as in the early case; the temperature fell, and the abdominal pain and tenesmus were diminished, but the frequency of bowel movements was only slightly affected. The reaction following the intravenous injection is mild. Out of three cases one felt a slight chilly sensation beginning forty minutes after injection and lasting only for ten minutes.

The intravenous injection should be made with caution. It is not improbable that serum may produce embolism under certain conditions.

So far as it was possible to gather from the available statistics on the rate of the mortality in bacillary dysentery treated with drugs as compared with the mortality in cases treated with serum, the following data show the observations of other investigators.

*a. Cases treated with drugs:*

Philippine General Hospital, (5) fiscal year 1912-1913—

Males, 191 with mortality of 17.8 per cent.

Females, 75 with mortality of 20 per cent.

Other hospitals (5)—

Japan, 16.5 to 30.2 per cent mortality.

Singapore (1902), 25.4 per cent mortality.

Ceylon (1903), 28.7 per cent mortality.

Hongkong (1902), 37.3 per cent mortality.

British New Guinea:

1902, 22.80 per cent mortality.

1903, 26.6 per cent mortality.

Egypt, 70 per cent mortality.(7)

b. Cases treated with serum:

Bahr (Fiji Island), 106 cases, 1.8 per cent mortality.(1)

Sandwith (England), 9 per cent mortality.(6)

Shiga (Japan), 9 per cent mortality.(4)

Willmore (Egypt) (1912-1913), 12 per cent mortality.(7)

My cases 20 (1 death), 5 per cent mortality.

In the above figures there is seen a considerable difference in mortality between the two methods of treatment. Undoubtedly the serum has done a great deal toward reducing the death rate.

While my investigations along this line have barely started, and my opportunities have so far been limited, I abstain from drawing definite conclusions for the present, although it cannot be doubted that the value of the specific treatment is strongly indicated as shown in the results hitherto attained. I hope, however, to carry out this work on a larger scale when ample opportunities are presented to me, from which definite conclusions may be drawn.

In conclusion I wish to express my obligations to Professor Ariston Bautista, chief of the Department of Medicine, for allowing me to carry out this work in his department; to Doctors Guerrero, Sison, Domingo, and Gutierrez for valuable suggestions during the course of treatment, and to Doctors Esquivel, Bañuelos, Hilario, Baltazar, and Concepcion for their coöperation. I am especially indebted to Dr. Otto Schöbl, of the Bureau of Science, who furnished me the serum and who made several bacteriological examinations of the stools for me. Also I have to thank him for his kindness in translating the German literature.

#### REFERENCES

- (1) BAHR, P. H. *Brit. Med. Journ.* (1914), 1, 294-296.
- (2) DOERR, R. In Kraus und Levaditi, *Handbuch der Technik und Methodik der Immunitäts-forschung*. Gustav Fischer, Jena (1909), 2, 164.
- (3) LUKIS, C. P. *Brit. Med. Journ.* (1913), 1, 1357-1359.
- (4) MANSON, P. *Tropical Diseases*. Fourth ed. Cassell and Company, London (1908), 436.
- (5) MUSGRAVE, W. E., and SISON, A. G. *Phil. Journ. Sci., Sec. B* (1914), 9, 241-251.
- (6) SANDWITH, F. N. *Lancet* (1914), 2, 637, 683, 731, 783.
- (7) WILLMORE. Cited by Bahr. See No. 1.

## ILLUSTRATIONS

**FIG. 1.** *Cases 8, 17, and 18.* Solid line, stools; broken line, temperature.  
One square is equivalent to one stool.

**2.** *Cases 12 and 13.* Solid line, stools; broken line, temperature. **One**  
square is equivalent to one stool.





## NOTE ON THE PORTAL OF ENTRY IN EXPERIMENTAL CHRONIC PULMONARY (SYSTEMIC) BLASTOMYCOSIS <sup>1</sup>

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That the usual portal of entry in systemic infections by the so-called *Blastomyces dermatitidis* of Gilchrist and Stokes is the respiratory tract itself has been held by most authors who have discussed the matter. This opinion has seemed justified by the usual absence of cutaneous foci or at least of any but apparently secondary lesions markedly in contrast with the usually extensive pulmonary involvement, evidently of long standing. In surprisingly few cases has a distinct history of cutaneous blastomycosis preceding the general invasion been obtained.

The possibility of error in a decision as to the point of origin in a generalized case, based on the evidence of lesions existing late in the disease, has been demonstrated by the findings in monkeys inoculated with this organism. In most of those that have died, especially when the inoculation was peripheral (subcutaneous or intramuscular), pulmonary lesions were found. In all but one animal these were essentially acute, appearing as granulomatous nodules or at the most as small circumscribed abscesses.

In one animal, in which death did not ensue until after a much longer period than usual, lesions developed that resembled those usually found at autopsy in human cases.

This monkey (No. 119), a full-grown, vigorous female, was inoculated on March 26, 1917, subcutaneously on the right side of the belly with 3 cubic centimeters of vigorous bouillon culture. A blastomycotic abscess developed within a few days at the inoculation site, and the inguinal lymph nodes on the same side became markedly enlarged. After about a month the abscess discharged, following which it retrogressed, so that on May 2 only a small, shallow ulcer remained. At this time it was noticed that the animal was well along in pregnancy. On June 10 she delivered an apparently normal young one. Though thin, the mother appeared to be in good health. The cutaneous lesion had practically disappeared, there being only a slight induration

<sup>1</sup> Received for publication December 18, 1917.

beneath the small scar. About July 1 observation was discontinued, the animal being apparently negative.

She did not gain weight, however, and the young one appeared to be undernourished. After three months more both animals were very thin, though in neither was any definite evidence of disease detected. In spite of special care, the mother continued slowly to lose weight and strength and on October 28 was no longer able to sit up. She died that night, seven months after inoculation and four and a half months after delivery. No cough was ever noted, there was no hemorrhage, and as is usual in blastomycosis in the monkey, there was no elevation of temperature on the few occasions when this was taken.

*Autopsy.* (October 29, 1917).—Autopsy disclosed a chronic pulmonary blastomycosis with cavitation very similar to that seen in man. The lobes of both lungs were adherent to each other, and lesions in adjacent lobes had become continuous in places. The visceral and parietal pleuræ were irregularly adherent, chiefly in the upper part, though the blastomycosis itself had not extended to them. The cavities were practically of equal extent in both lungs, involving considerable areas and communicating with the bronchi. They were lined with gray necrotic material of unpleasant odor. The walls were of variable thickness, fibrosed in places, but for the most part actively granular.

The other organs presented no perceptible blastomycotic focus. The liver was somewhat fatty, the spleen was a trifle enlarged and slightly congested, and the kidneys showed a fairly marked cloudy swelling. One small inguinal lymph node on the right side, no larger than a small pea, contained a single drop of pus.

Material from the lungs, in fresh and stained smears and in tissue sections, showed abundant organisms, together with the tissue changes typical of this infection. Cultures were not made, there being no possible doubt as to the identity of the organism. In fresh preparations of material from a cut surface of the spleen occasional blastomycetes were found, though there was no indication of local activity on their part. Preparations from other abdominal viscera were negative for the organisms. A few were found in the pus from the right inguinal lymph node.

#### DISCUSSION

The lesion described was the only instance of chronic pulmonary infection, similar to that found in human cases, that has developed in a total of about 40 monkeys variously inoculated with this organism. In all other instances that have proved

fatal and in which pulmonary lesions were found these were comparatively acute and were either small granulomatous nodules or small circumscribed abscesses. Metastasis to the lungs has been almost constant and often very extensive, particularly in the monkeys inoculated peripherally; metastases in other viscera have, on the contrary, been comparatively uncommon. In a few cases it has happened, in animals that have been subjected to unfavorable influences, that the focus at the point of subcutaneous inoculation proved comparatively insignificant, in contrast to the extensive pulmonary involvement.

In the animal under discussion pulmonary metastases from the original subcutaneous focus developed, possibly after a period of quiescence, in spite of the subsidence of the peripheral lesion. Whether the metastases would have progressed to a fatal issue in an ordinary animal without the physiological responsibilities of maternity cannot be said, though it seems to me likely that, in spite of the fact that the monkey is more susceptible to this infection than any other laboratory animal, the secondary foci might have been overcome as was the subcutaneous lesion.

The factors that may have influenced the development are for the present discussion incidental to the fact. The point is that had the animal been seen first after the subsidence of the lesion at the point of inoculation, where only a clean, inconspicuous scar remained, there would obviously have been little possibility of recognizing that as the portal of entry of the infection.

It is evident that should similar pulmonary metastasis from a primary subcutaneous or other focus occur in a human case, with subsequent disappearance of the original peripheral lesion, which need never have been particularly large or striking, the onset of the systemic disease might well appear to coincide with or to follow a cold or other temporary disturbance, as, for example, was the history in the majority of the cases reviewed by Stober.<sup>2</sup> This would serve to concentrate attention unduly upon the respiratory tract as the point of origin. In view of the long latency possible in this infection this exacerbation might, as in pulmonary tuberculosis, be at a date comparatively remote from the time of primary invasion.

#### SUMMARY

A full-grown monkey, later found to be pregnant, was inoculated subcutaneously with the so-called *Blastomyces dermatitidis*, a small abscess resulting. After two and a half months,

<sup>2</sup> Stober, A, M., *Arch. Int. Med.* (1914), 13, 509.



at which time the abscess had healed, she delivered a healthy young one. Neither thrived; the mother slowly became thinner and died of exhaustion four and one-half months after delivery. Autopsy revealed a bilateral, chronic pulmonary blastomycosis similar to that usually found in human cases. From the evidence presented during the last five months of life, or elicited by autopsy, the erroneous conclusion that the infection was primarily pulmonary could hardly have been avoided. By analogy it seems possible for systemic blastomycosis in man to be similarly inaugurated, perhaps at a considerable time before, by a temporary or inconspicuous peripheral lesion. Therefore, though it is highly probable that the respiratory tract may more or less frequently be primarily affected, the conclusion that this is the constant or even the usual portal of entry is hardly to be justified on the basis of imperfect history or of late clinical and autopsy findings.

## PRESERVATION OF CHOLERA STOOL SPECIMENS FOR DELAYED BACTERIOLOGIC EXAMINATION<sup>1</sup>

By C. S. PANGANIBAN and OTTO SCHÖBL

(From the Serum Section of the Biological Laboratory, Bureau of Science,  
Manila)

The importance of bacteriologic diagnosis of Asiatic cholera is generally appreciated by practical sanitarians. Its importance increased considerably since it became generally known that by means of bacteriological examination of fæces the detection of cholera carriers could be made possible. As reliable as the laboratory procedure is to detect the cholera vibrios when present in the fæces even in small numbers, the rapid disappearance of cholera vibrios in the fæces exposed to higher temperature particularly in presence of other intestinal bacteria is no doubt responsible for the failures in delayed examinations. It would, therefore, be of some value if a method could be devised to preserve the specimen for such a length of time as is necessary to transport it from the source to a laboratory equipped for bacteriological diagnosis of cholera.

Several ways suggest themselves from previous experiences of others and our own.

1. The method of preserving fæces with glycerin suggested by Teague<sup>2</sup> for delayed examination of typhoid stools.

2. Sodium chloride solution. It was found in previous researches concerning the survival of cholera vibrios in water that cholera vibrios remain alive in sea water for a considerable length of time—in our particular experiment, between 106 and 120 days.<sup>3</sup> This experience could be probably made use of.

3. Bile. This medium, first recommended by Otolenghi, possesses selective properties of high degree with regard to cholera vibrios. Cholera vibrios in pure culture survive in bile for an indefinite length of time. This fact was referred to briefly in a previous communication<sup>4</sup> and has been utilized

<sup>1</sup> Received for publication February 21, 1918.

<sup>2</sup> Teague, Oscar, and Clurman, A. W., *Journ Inf. Dis.* (1916), 18.  
Schöbl, Otto, *Phil. Journ. Sci., Sec. B.* (1914), 9, 479.

<sup>4</sup> Idem, *Journ. Inf. Dis.* (1916), 18, 307.

since by keeping stock cultures of cholera growing in bile medium.

The present investigation consists of three experiments, namely, with glycerin, with sodium chloride solutions, and with bile, respectively. Although these experiments have not been carried out simultaneously, the salt solution used in each of the experiments serves as *tertium comparationis* and allows fair comparison of the results of the three groups of experiments.

#### EXPERIMENT I. GLYCERIN

About 3 grams of stools were ground up in 30 cubic centimeters of 0.6 per cent salt solution. The mixture was filtered through cheesecloth. Twenty cubic centimeters of 0.6 per cent salt solution were added to two 24-hour old cultures of cholera vibrios, the cultures were washed, and the suspension was added to the filtrate of the stool mentioned above. Thus prepared artificial cholera-stool emulsion was thoroughly shaken in a flask containing glass beads.

Five cubic centimeters of 0.6 per cent salt solution and 40 per cent, 50 per cent, and 60 per cent glycerin, respectively, were put into four separate sterile test tubes, and to each one of these were added 5 cubic centimeters of the above suspension of fæces. The tubes were then thoroughly shaken and left at room temperature (32° C.). One peptone water culture was planted from each of the four tubes immediately by transferring three loopfuls of their content. After twenty-four hours' incubation Dieudonné's plates were made from the peptone cultures. At the end of twenty-four hours' incubation the colonies that developed on the plates were examined. Smears were made, and microscopic agglutination was performed.

The three tubes containing the mixture of artificial cholera fæces and glycerin in various strength of percentage as well as the fourth tube containing no glycerin but only salt solution were examined daily for the presence or the absence of cholera vibrios by the procedure just described.

The results of this experiment (Table I) show that glycerin has no preserving action for cholera vibrios. In 20 per cent and in 25 per cent concentrations the cholera vibrios survived four days only, while in the tube containing 30 per cent glycerin no cholera vibrios could be found on the fourth and the subsequent days. These findings are in accord with those obtained by Ruediger, who made similar observations while testing the germicidal action of glycerin.

## EXPERIMENTS II AND III. SALT SOLUTION AND OX BILE

Five cubic centimeters of sterile sodium chloride solution, in concentration of 0.5 per cent, 1 per cent, 2 per cent, 5 per cent, 10 per cent, 15 per cent, 20 per cent, 25 per cent, and 30 per cent, were placed into a set of sterile test tubes, and 5 cubic centimeters of 50 per cent, 75 per cent, and pure ox bile were put into the tubes of the second set. In both of these sets one tube containing 5 cubic centimeters of sterile normal salt solution was used as a control.

About 10 grams of normal stool were ground up in 100 cubic centimeters of sterile normal salt solution, and the mixture was filtered through cheesecloth. Ten cubic centimeters of sterile normal salt solution were added to each of four 24-hour-old agar cultures of cholera vibrio, which were then washed, and the suspension was added to the filtered emulsion of the stool. The final mixture of cholera vibrios and stool was thoroughly shaken in flasks containing glass beads. Five cubic centimeters of this suspension were then added to each one of the tubes containing varying concentrations of salt solution and ox bile, respectively. The tubes, after the addition of cholera stool suspension, were thoroughly shaken and were kept at room temperature (32° C.). Immediately after mixing, one peptone water culture was planted from each of the tubes by transferring three loopfuls of the stool emulsion. After twenty-four hours' incubation Dieudonné's plates were planted from the peptone culture. At the end of another twenty-four hours' incubation the colonies that developed were examined microscopically. Smears were made, and microscopic agglutination was performed.

Similarly peptone water cultures, Dieudonné's plate, and microscopic examinations of the colonies were made every day during the first week, after which time examinations were made once a week only. It is evident from Table II that sodium chloride preserved the cholera fæces successfully for the period of five weeks, at least, in concentration of from 0.5 to 5 per cent. In concentration higher than 5 per cent the vibrios could not be found after five and four days, respectively.

In Table III the results of the experiment, in which bile was used as preservative, are tabulated. It shows successful preservation of cholera fæces in dilution of 50 per cent, 75 per cent, and 100 per cent of bile with normal salt solution as a control.

In the previous experiments the sodium chloride solution and ox bile gave equally good results as preservatives of cholera stools for delayed examination when the amount of cholera





TABLE III.—*Showing the preserving action of ox bile on cholera vibrios.*

DIEUDONNÉ'S PLATE.

Concentration of bile in H <sub>2</sub> O.	In days.							In weeks.				
	0	1	2	3	4	5	6	7	2	3	4	5
50 per cent .....	+	+	+	+	+	+	+	+	+	+	+	+
75 per cent .....	+	+	+	+	+		+	+	+		+	+
Pure bile .....	+	+	+	+	+		+	+	+	+	+	+
Control (normal salt solution) .....	+	+	+	+	+	+	+	+	+	+	+	+

TABLE IV.—*Showing the preserving action of 1 per cent salt solution on cholera vibrios.*

DIEUDONNÉ'S PLATE.

Amount of cholera vibrios in terms of loop.	In days.							In weeks.						
	0	1	2	3	4	5	6	7	2	3	4	5	6	7
2 .....	+	+	+											
1 .....	+	+	+											
0.5 .....	+	+	+											
0.1 .....	+	+	+											
0.01 .....	+	+	+											
0.005 .....	+	+	+											
0.001 .....	+	+	+											
0.0005 .....	+	+	+											
0.0001 .....	+	+	+											
0.00005 .....	+	+	+											
0.00001 .....	+	+	+											
0.000005 .....	+	+	+											
0.000001 .....	+	+	+											
0.0000005 .....	+	+	+											
0.0000001 .....	+	+	+											
0.00000005 .....	+	+	+											
0.00000001 .....	+	+	+											
0.000000005 .....	+	+	+											
0.000000001 .....	+	+	+											

<sup>a</sup> Not tested after two days.     <sup>b</sup> Not tested after three weeks.



## REVIEWS

A Practical Text-book | of | Infection, Immunity | and Specific Therapy | with special reference to immunologic technic | by | John A. Kolmer, M. D., Dr. P. H., M. Sc. | [4 lines] | with an introduction by | Allen J. Smith, M. D., Sc. D., LL. D. | [1 line] | with 147 original illustrations, 46 in colors | by Erwin F. Faber | [1 line] | second edition, thoroughly revised | Philadelphia and London | W. B. Saunders Company | 1917. Cloth, pp. i-xiii—1-978. Price \$7.00 net. Half morocco, \$8.50.

The following is from the preface to the second edition:

Additions and alterations have been made throughout; special attention has been given the subject of local infection; the Schick toxin test for immunity in diphtheria and active immunization in diphtheria and active immunization in diphtheria with toxin-antitoxin mixtures; complement-fixation in tuberculosis and other bacterial infections and a quantitative Wassermann reaction based upon my studies with the co-operation and assistance of Dr. Claude P. Brown, Dr. Toitsu Matsunami, and Dr. Berta Meine, aiming to standardize this important test. The chapters on anaphylaxis have been revised and particular attention given the subject of anaphylactic skin reactions. Lange's colloidal gold reaction has been included. The chapter on the treatment of various infections with bacterial vaccines has been enlarged and the non-specific activity of bacterial vaccines discussed. The section on the treatment of certain of the acute infectious diseases, and particularly acute anterior poliomyelitis, with the serum of convalescents and normal persons has been amplified; blood transfusion has been included. Special attention has been devoted to the chapter on Chemotherapy, and the results of the studies of Dr. Jay F. Schamberg, Dr. George Raiziss, and the author bearing upon the toxicity of salvarsan and its congeners and the reactions following their administration have been included and discussed. The subject of Bacterial Chemotherapy, which promises much in the future, has been amplified from the theoretical and technical viewpoints.

American Addresses | by | Sir Berkeley Moynihan, M. S., F. R. C. S. | [ornament] | Philadelphia and London | W. B. Saunders Company | 1917. Cloth, pp. 1-143. Price \$1.75.

From the preface we quote the following:

The papers included in this volume were read in Chicago and elsewhere during October and November, 1917. I hope they may help my American colleagues to some appreciation of the causes and conditions of the war, and afford some help to them in their treatment of the many phases of surgical diseases with which they will be called upon to deal.

The papers represent not only my own views and experience, but those of others also. In forming my opinions upon the several matters dis-



cussed I have received very great help from the many consultations and discussions I have had with many of my friends in the different war zones of France and England.

The book contains the following chapters: The causes of the war; gunshot wounds and their treatment; wounds of the knee-joint; on injuries to the peripheral nerves and their treatment; and gunshot wounds of the lungs and pleura.

**Tumors of the Nervus Acusticus | and the Syndrome of the Cerebellopontile Angle |** By | Harvey Cushing, M. D. | [five lines] | Illustrated | Philadelphia and London | W. B. Saunders Company | 1917. Pp. i-viii—1-296. Cloth, \$5.00 net.

From the preface we quote the following:

In the course of preparation of a monograph dealing with a series of meningeal fibro-endotheliomata, a careful review was necessitated of the pathological as well as the clinical aspects of these interesting tumors. They have their point of origin in certain definite regions, and a tentative subdivision had been made of those arising from the spinal meninges, those from the basilar meninges, and those from the superior envelopes of the brain.

It was apparent that the spinal and basilar lesions usually arose from the meninges at the point of exit of a spinal or cerebral nerve root, and it was anticipated that many of the tumors of the cerebellopontile angle which involve the acoustic nerve would be included in the series, for the majority of them had previously been diagnosed from their gross appearance, though admittedly with some reservation on histological grounds, as endotheliomata.

Hesitation was felt in regard to the inclusion in the series of some of the spinal cord tumors, and these doubts became intensified when the lateral recess tumors came to be assembled and closely inspected.

A thorough rehearsal of the material at hand, comprising twenty-nine histologically certified cases, together with a much larger number of probable though unverified ones, which nevertheless were useful from the standpoint of their clinical data, so clarified many obscure matters relating to these peculiar and unmistakable tumors of the VIIIth nerve that they have been made the subject of this separate study, and a report upon the 60 endotheliomata proper must await its turn. Unquestionably the acoustic tumors are most distinctive growths and such relationship as they have to the meningeal tumors occurring in the lateral recess will be pointed out in its proper place.

Some important monographs on the subject have already been published, of which Folke Henschen's Inaugural Dissertation, 1910, is the most noteworthy, but in all of them the various tumors of the cerebellopontile angle have been incorporated, whereas the acoustic neuromas will alone occupy our attention to the exclusion of other tumors of the recess except in so far as they are of interest from the standpoint of differential diagnosis.

## PROCEEDINGS OF THE MANILA MEDICAL SOCIETY

REGULAR MONTHLY MEETING, APRIL 1, 1918

### MINUTES OF THE MANILA MEDICAL SOCIETY

The regular meeting of the Manila Medical Society was held at the College of Medicine and Surgery, April 1, 1918, at 9 in the evening.

One visitor and seven members were present.

The minutes of the previous meeting were read and approved as read.

On the recommendation of the council, the application of Drs. Herminio Velarde y Esquivel and Walfrido de Leon for active membership was ratified by the society.

In the absence of Dr. J. A. Johnston, his interesting paper on Twenty-five Years of Laboratory Work was read by Dr. R. B. Gibson.

Dr. José S. Hilario followed with a paper entitled the Widal Test at Different Stages of Typhoid Fever, which was discussed by Doctors Gonzales, Vincent, and de la Paz.

Dr. Isabelo Concepción presented a paper on Analysis of Normal Filipino Urine. This paper was discussed by Doctors Gibson, Gonzales, Hilario, and de la Paz.

Doctor Esquivel, visitor, next demonstrated a case of splenomegaly.

The chairman announced that by action of the council the meeting of the society during May, June, and July would be suspended on account of warm weather and the next meeting would be in August.

The meeting was adjourned at 11.15.

D. DE LA PAZ,  
*Secretary-Treasurer,*  
*Manila Medical Society.*

### SCIENTIFIC PROGRAM

#### TWENTY-FIVE YEARS OF LABORATORY WORK

By DR. JOHN A. JOHNSTON

An interesting contrast of the workers, methods, and laboratories of twenty-five years ago with those of to-day.

THE GRUBLER-WIDAL TEST AT DIFFERENT STAGES OF  
TYPHOID FEVER

By DR. J. S. HILARIO

The diagnosis of infectious diseases by means of agglutination test is one of the most useful means that is available to recognize infectious diseases such as typhoid fever. The present paper gives the findings in 818 cases submitted to the Grubler-Widal test, of which 607 were typhoid, 154 were doubtful or suspected typhoid, and 77 were other diseases. Of 818 tests performed, 404 were positive, giving a percentage of 56.8 positive. The test was performed with *Bacillus typhosus*, *B. paratyphosus-A*, and *B. paratyphosus-B* simultaneously on each individual case. During the first week of typhoid infection the percentage positive found was 42.3; during the second week, 65.4; during the third week, 65.9; during the fourth week, 72.8; during the seventh week, 100. A series of 440 cases submitted to simultaneous tests with three organisms of the typhoid-paratyphoid group has given the following results: With *Bacillus typhosus* the positive tests were 52.04 per cent; with *B. paratyphosus-B*, 30.04 per cent; with *B. paratyphosus-A*, 9.77 per cent. In a series of 226 positive tests the presence of group-agglutinations among members of the typhoid-paratyphoid group is shown by the following: Group-agglutination between *typhosus* and *paratyphosus-B* was found in 44.6 per cent; between *typhosus* and *paratyphosus-A* in 5.3 per cent; and between *typhosus*, *paratyphosus-A* and *paratyphosus-B* in 6.6 per cent. As to diseases other than typhoid the results of the test made were as follows: Of 103 cases of undetermined fever 43 were positive for *typhosus*, giving an average of 41 per cent; of 4 cases of malaria, 1 was positive for *typhosus* and 1 for *paratyphosus-B*; of 3 cases of pneumonia, 1 was positive for *typhosus*; of 2 cases of meningitis, 1 was positive for *typhosus*; of 1 case of pulmonary tuberculosis, 1 was positive for *typhosus*; of 2 cases of bronchopneumonia, both were positive for *typhosus*; and 1 case of dysentery and 1 case of puerperal fever were also positive for *typhosus*. In view of the above data, the following conclusions may be established:

(1) Infection with *Bacillus typhosus* is the most frequent in Manila, while paratyphoid infections also occur, although not so frequently, the infection with *paratyphosus-B* being more frequent than that with *paratyphosus-A*, which is rare.

(2) Typhoid-paratyphoid-B group-agglutinations are the com-



monest, representing about 50 per cent of all the group-agglutinations observed.

#### ANALYSIS OF NORMAL FILIPINO URINE

By DR. ISABELO CONCEPCIÓN

Analyses were reported of the twenty-four hour urines of students, laboratory helpers, prisoners, and hospital servants. Characteristic findings for Filipinos are a small volume, low total nitrogen (3.05 to 12.63 grams), a low percentage of urea nitrogen with reference to the total nitrogen, low uric acid (average 0.376 gram), and low chlorides (average 5.86 grams as sodium chloride). The ammonia, the creatinine, the undetermined nitrogen, and the ratios nitrogen phosphoric acid and nitrogen sulphur are in accord with the low nitrogen values of the Filipino dietary.

R. B. GIBSON,

*Editor of the Proceedings,*

*Manila Medical Society.*

The Philippine Islands Medical Association held a joint scientific and social session February 4 to 9, inclusive, with the IV Asamblea Regional de Medicos y Farmaceuticos de Filipinas. The scientific proceedings will be published with those of that organization. At the close of the scientific sessions, the business meeting of the association was adjourned to follow some meeting of the Manila Medical Society in the near future at the call of the secretary-treasurer, at which time the election of officers was to be held.

#### MINUTES OF THE PHILIPPINE ISLANDS MEDICAL ASSOCIATION

Postponed business meeting, April 1, 1918.

The meeting was called to order at the close of the regular monthly meeting of the Manila Medical Society, the vice-president, Doctor Calderon, presiding.

The report of the nominating committee was presented and on motion, duly seconded, the secretary-treasurer was instructed to cast the ballot for the association for the following nominees:

President:

Dr. Fernando Calderon.

Vice-presidents:

Dr. E. S. Ruth.

Dr. Jesus Gonzalez.

Councilor:

Dr. Daniel de la Paz.

There being no further business, the meeting was adjourned.

R. B. GIBSON,

*Secretary-Treasurer,*

*Philippine Islands Medical Association.*





# THE PHILIPPINE JOURNAL OF SCIENCE

## B. TROPICAL MEDICINE

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### ENDEMIC MALARIA IN THE PHILIPPINE ISLANDS AS A MILITARY PROBLEM<sup>1</sup>

By FRANK G. HAUGHWOÛT

*(Professor of Protozoölogy and Chief, Department of Parasitology, College of Medicine and Surgery, University of the Philippines.)*

New institutions in a developing country almost always bring new problems of a troublesome and perplexing nature. The degree of success that attends efforts to solve these problems is in direct proportion to the foreknowledge and farsightedness of those who are called upon to cope with them. With the recruiting of large bodies of men, and their organization into a military unit, problems of vital concern to those of the medical and allied professions charged with safeguarding the health of the military force and of the public in general are almost certain to develop. Closely allied with these problems are our obligations, not only to our own community, but to others with whom we come in contact, and we should be culpably negligent should we fail to bear them well in mind and exert ourselves to meet them.

In this paper I shall deal with a single phase of one of these problems only—the malaria problem—and I shall try to present an outline of some of the recent work on the subject, which I consider pertinent to the question. Even at the risk of being termed an alarmist, I shall try to show that aside from the peril of cholera, typhoid, the dysenteries, and smallpox malaria constitutes one of the gravest medial problems, if not the gravest, with which an army medical corps has to deal in connection with organization work in the tropics.

Another blood parasite that is almost equally to be dreaded

<sup>1</sup> Read before the Manila Medical Society, August 5, 1918.

is the nematode *Filaria bancrofti*. Its transmitting agent, *Culex fatigans*, is common in Manila and the vicinity and is of wide general distribution.

There was recently published a newspaper article<sup>2</sup> based on figures compiled by the Bureau of Health and issued on June 28, 1918, in which attention was called to five diseases that the Philippine Islands must fight. The statistics showing the deaths from these five diseases during the last decade are here given:

Smallpox	21,978
Beriberi	47,052
Cholera	52,804
Tuberculosis	192,841
Malaria	247,675

These figures furnish an interesting illustration of the psychology of the masses. Generally, two or three cases of cholera or smallpox will suffice to reduce the average community to a state of mind bordering on hysterics, and to evoke a prompt and strong reaction on the part of the local health authorities.

The constant presence of beriberi and tuberculosis brings about another strong reaction in the form of the establishment of societies, organized to supplement the work of the health authorities. This is quite as it should be; but, meanwhile, the effective partnership of *Anopheles* and *Plasmodium* shows in ten years a ledger credit in this country of nearly a quarter of a million human lives unostentatiously, but none the less effectively, terminated by malarial fevers and their complications and sequelæ.

Many prominent capitalists and business men of the Philippine Islands consider malaria one of the most serious impediments to the commercial and economic development of the country that exists. Notwithstanding all this, no one seems ever to have dreamed of founding an antimalaria society, or of going out on the highways and byways to solicit funds for the suppression of malaria or for the establishment of institutions for the treatment of those who have fallen victims to it.

The distribution of malaria in the Philippine Islands, as shown by the records of the Bureau of Health, is a matter of some interest, especially when compared with two other serious diseases—cholera and tuberculosis. I quote from the published records of the Bureau of Health,<sup>(24)</sup> as to the causes of death during the year 1916.

<sup>2</sup> *Manila Daily Bulletin* (July 1, 1918), 6.

TABLE I.—Deaths from cholera, tuberculosis, and malaria in the Philippine Islands during 1916.

Province.	Malaria.	Malarial cachexia.	Cholera.	Tuberculosis of the lungs.	Tuberculosis of other organs.
Abra .....	621	9		145	2
Agusan .....	142	10		15	2
Albay .....	730	69	631	731	78
Ambos Camarines .....	1,007	66	895	709	87
Antique .....	240		47	489	20
Bataan .....	266	99	153	110	18
Batanes .....	6	1		12	2
Batangas .....	786	39	75	608	40
Bohol .....	109	11	27	408	101
Bulacan .....	547	29	808	941	23
Cagayan .....	903	17		351	30
Capiz .....	727	54	97	727	61
Cavite .....	691	130	235	250	26
Cebu .....	871	41	12	902	108
Ilocos Norte .....	516	64		232	91
Ilocos Sur .....	831	3		310	16
Iloilo .....	880	166	1,653	1,476	102
Isabela .....	523	34		211	16
Laguna .....	713	127		560	42
Leyte .....					
Mindoro .....	510		12	222	2
Misamis .....	669	4	228	318	37
Nueva Ecija .....	525	70	9	477	31
Nueva Vizcaya .....	127	139		90	2
Occidental Negros .....	1,049	154	1,293	1,021	68
Oriental Negros .....	1,067	6		229	63
Palawan .....	66	14		34	9
Pampanga .....	593	129	203	647	19
Pangasinan .....	3,119	197	2	1,476	80
Rizal .....	276	79	377	415	111
Romblon .....	146	31	138	67	11
Samar .....	525	13	256	218	104
Sorsogon .....					
Surigao .....	218			33	13
Tarlac .....	391	50	1	412	15
Tayabas .....	790	158	10	702	43
Union .....	321	104		297	9
Zambales .....	5	122	31	269	9
	21,506	2,239	7,193	16,114	1,491

These figures will be seen to total as follows:

Cholera	7,193
Tuberculosis	17,605
Malaria	23,745

In other words, the deaths from cholera and tuberculosis, combined, were only 1,053 in excess of those reported as being caused by malaria and malarial cachexia.



Two entire provinces—Leyte and Sorsogon—are unreported; and other questions, such as the accuracy of diagnosis in all cases and the difficulty of securing correct reports from the wild tribes and the sparsely settled and inaccessible districts in the country, are apparently not considered. So that, if a precise study of the incidence and distribution of malaria in the Philippine Islands were to be made, the statistics given above would have to be carefully analyzed. In the main, however, the figures probably give a fair idea of the proportion malaria bears to the other two most dreaded diseases, and of its relative extent and distribution throughout the Islands.

The death rate from malaria, as evidenced by these statistics, is startling enough; but there is another factor which has a direct bearing upon the effects of malaria on the population of a country, which seems to be totally neglected although, on reflection, it must appeal to every physician practicing in the tropics. That factor is furnished by death of the fœtus in utero as a result of a supervening attack of malaria suffered by the mother. Cases in which the pregnant woman aborts during an attack of malaria are all too familiar; under treatment she may survive, but her child is lost. I can give no figures as to the extent of this; however, I can cite some interesting data collected by the sanitary authorities of Burma<sup>(3)</sup> based on the child population of all the districts in that province.

The province was very thoroughly canvassed and a large number of persons examined. The table gives comparative figures of the child population, under 10 years of age, per hundred married females of child-bearing age (15 to 40 years). It is probably a reliable statement of the relative proportional distribution of the child population and of the relative productive capacity of the female population in the different districts. Inspection of the figures will show that the child population is materially reduced in malarious districts as contrasted with those that are nonmalarious. The table follows:

TABLE II.—*Child population of Burma.*

Malarious Districts.	Children.	Relatively nonmalarious districts.	Children.
Kyaukee.....	186	Magwe.....	245
Mandalay.....	188	Sagaing.....	244
Thayetmye.....	198	Meiktila.....	244
Prome.....	198	Myingyan.....	240
Yamethin.....	208	Lower Chindwin.....	236
Ma-ubin.....	210	Pakokku.....	223
Schwebo.....	218		

All this should help us to form an opinion as to the extent of the fundamental problem that will be presented by the importation into Manila and its vicinity of several thousand men, drawn from the various provinces in the Islands<sup>3</sup> and destined to form the ranks of a military unit. That a not inconsiderable proportion of these men will prove to be carriers of the malarial parasite is a probability that it will not be safe to disregard; that, as such, they will constitute a source of danger naturally follows.

First, they will be a source of danger locally; for Manila harbors mosquitoes of the malaria-transmitting type, and local conditions show a tendency to become increasingly favorable to their development. However, it is not particularly my purpose to deal with the Manila problem in its especial reference to the danger to the civilian population of that city.

Secondly, they will constitute a serious source of danger to their camp mates wherever an encampment may be established. Before such an encampment is established a careful preliminary survey of the site proposed should be made by thoroughly competent men. Neglect to do so may give rise to an exceedingly discouraging and depressing experience, especially if the camp be located in proximity to a native village where malaria is almost certain to be endemic, if not epidemic. In this connection Hoffman(14) lays stress on the value to the army medical corps of the services of an expert entomologist.

Lastly—and herein lies a duty to those with whom they may be called upon to serve, and that duty involves a heavy obligation—there is the danger to troops from other countries, should the fortunes of war send the Philippine contingent to associate with them. Unless the work of weeding out or sterilizing malaria carriers is conscientiously and effectively carried out before leaving the home country, the troops may do far more harm than good. They are certain to be the bearers of new and malignant strains(5) of the parasites. It would not be a new experience, for other troops have come from the Far East bearing similar indirect aids to the enemy.

Important and destructive as they are, the intestinal parasitic diseases constitute a more controllable problem among troops at war than does malaria. Trench hygiene will, within certain bounds, control the diseases conveyed by contaminative methods;

\* Attention is particularly directed to the returns from Ambos Camarines, Oriental and Occidental Negros, and Pangasinan, which report deaths caused by malaria varying from 1,000 to more than 3,000 during 1916.

but in a bullet-swept country, dotted with water-filled shell holes, or in places still more favorable to the development of *Anopheles* there is little opportunity to control malaria through the eradication of mosquitoes. Recourse must be had to quinine prophylaxis—the efficacy of which in all cases is open to doubt—or to the elimination of carriers of the parasite. As suggested in a previous paragraph, much can be done in the home country to minimize the danger, before the troops leave for the front.<sup>4</sup>

The formidable problem presented by malaria on the battle line and at the base has been well borne in on the military surgeons of all countries during the present struggle. The protean nature of the disease itself is perfectly familiar to every physician. Carles<sup>(4)</sup> places malaria in the same class with entamœbiasis and syphilis, as a disease eminently chronic and characterized by frequent revivals of manifestations, which requires persistent treatment over years, as is the case with syphilis and entamœbiasis. He considers every man suffering from grave malarial infection disqualified for duty for a period of from six months to a year. In such cases every return to a fatiguing occupation brings about a new exacerbation and a general decline in health as a consequence.

Gill<sup>(12)</sup> has recently made an interesting inquiry into malaria as it exists in the Indian army. In his opinion the greater part of the sickness in the native Indian army is attributable, either directly or indirectly, to malaria. In the Punjab, for instance, one of the great recruiting grounds, the recruits come from a population that is widely infected every year, and in some years grossly infected. Examination of the men in the ranks as well as of the recruits has shown that a large proportion of them,

<sup>4</sup>The problem of prophylaxis against malaria in tropical countries presents difficulties that are not met in most temperate climates. The importance of the elimination of the anopheline mosquito is by no means to be discounted, for it is of incalculable value in communities peopled by persons of intelligence and having a well-organized health service. But in sections of a tropical country, to which a health service has not been extended, the problem is different. In such places the eradication of breeding places of the mosquito, except under the most favorable natural conditions, is virtually an impossibility. No aid in the carrying out of antimosquito work can be expected from semicivilized populations. Unless it is possible to enforce what virtually amounts to military discipline, the systematic use of quinine as a prophylactic cannot be carried out. The screening of houses or even of beds cannot even be dreamed of. Of all the health problems met in such communities, malaria is by far the most formidable. *The eradication of malaria under these circumstances, still remains a laboratory problem and will so remain until means are discovered for killing the gametocyte in the human body.*



while seemingly in perfect health, show enlarged spleens. Cases of this character may never enter the hospital at all, or they may enter to receive treatment for some other ailment contingent upon chronic malaria, although not so recorded on the hospital returns.

Gill goes on to state that the problem of the prevention of malaria in war resolves itself largely into the prevention of malaria, in cantonments at the very least, in times of peace—this in order that the army may confidently take the field free of autogenous infection, especially if conditions there be favorable to the spread of malarial infection.

Gill has shown the conditions met in India by the recruiting staff. The sequel at the front is shown by Woodcock.<sup>(36)</sup> I quote from the latter author (p. 300):

Until July there was scarcely any malaria, but during that month it began to increase. The worst months were September and October. Parasites were found in forty-three per cent of the cases, and especially among the Indians; quite five or six per cent in addition were obviously malarial bloods, although no parasites could be found. The findings are summarized in Table IV.

TABLE IV.—CASES OF MALARIA, AUGUST 1 TO NOVEMBER 30 INCLUSIVE.

	Examinations made.	Number positive.	Malignant tertian.		Benign tertian.		Quartan.	
				<i>P. ct.</i>		<i>P. ct.</i>		<i>P. ct.</i>
British .....	463	178	66	37	112	63	0	0
Indians and Egyptian Labour Corps .....	374	192	153	79.7	37	20.2	2	1
Totals .....	837	370	219	59.2	149	40	2	0.5

[The form of this table differs slightly from the original, but the numbers and headings are unchanged.—EDITOR.]

Among the British, benign tertian was twice as frequent as malignant tertian (pernicious), but among the Indians and Egyptian Labour Corps the malignant form was by far the most common. The rarity of quartan cases was noteworthy, only two being met with, both occurring in Indians. I was struck by the scantiness of the parasites in quite a number of the benign tertian cases among the British. Probably insufficient prophylactic dosage of quinine was responsible, checking but not completely inhibiting the development of the parasites.

In all the British malignant cases, the parasites (in the ring-form) were frequent or numerous, whereas the contrary was often the case among the Indians. On two or three occasions, malignant parasites very bacilliform in character were seen, but a few typical rings could always be found by searching. *Crescents were never found in the British cases, as nearly all of these were new infections.* [The italics are mine, F. G. H.]



Of course these figures would require careful analysis if one were to establish a proportion of infections among the British troops as having been derived from the Indians and the Egyptians. The predominance of benign over malignant infections in the British troops might then be found to be due to the comparative scarcity of anophelines of the species suitable for transmitting the malignant parasites. The fact remains, however, that the Indians and Egyptians seem to have been preponderant as harborers of the malignant parasite in a late stage of its development; and, even if they were not responsible for the incidence of the disease among the British, they not only helped to fill the hospitals, but also caused a corresponding decrease in the efficiency of their unit.

The baleful influence of malaria is by no means restricted to the medical side of the military hospital. Vandenbosche<sup>(34)</sup> has made a study in Salonika of the effects of malaria on wounds. Many of the phenomena he records are more or less familiar to physicians who have practiced in malarious regions. He states that even slight wounds will bring on an attack of malaria in patients who have previously suffered from the disease and that operations frequently have the same effect, the paroxysm occurring from one to six days after the operation. Such relapses, according to Vandenbosche, are prone to follow chloroform anæsthesia, and he recommends the substitution of ether. From my own experience in connection with a series of recent surgical cases at St. Luke's Hospital, Manila, in collaboration with Dr. A. F. Coutant, I incline to the opinion that relapse may also follow prolonged ether anæsthesia.

Vandenbosche's observations bear out those of Gill: that, in intensely malarious regions, latent malaria is all the more dangerous since in those districts persons, who to all appearances are perfectly healthy, may yet be gametocyte carriers, even though they had manifested no symptoms of the disease.

In his rounds of the hospitals in Salonika, Vandenbosche was able to observe most diverse hæmorrhagic phenomena in malarious subjects. These took the form of epistaxis which was occasionally fatal, hæmoptysis, hæmaturia, and petechial and ecchymotic patches. He regards every malarial subject as a potential "bleeder" and advises that surgical procedures be undertaken only under the strictest precautions. He points out the tendency of the disease to blur the clinical picture produced by a wound or by other complications, and admonishes surgeons to remember the possibility of malaria before enlarging or opening a wound on elevation of temperature. Malarial gangrene

he mentions as a rare but possible complication. He also speaks of cases of malaria that simulated appendicitis so closely as to bring the patient to the operating table.

The frequent failure of protozoan parasites to play the game according to the rules is a constant source of perplexity to physicians and parasitologists. It has long been an accepted rule that an attack of malaria might be expected to develop in from ten to fifteen days after the bite of an infected anopheline. Garin<sup>(10)</sup> dissents from this, and cites his evidence to the contrary, secured through a study of conditions in Macedonia. He produces evidence of a clinical and microscopical nature, tending to show that persons may become infected with malaria but remain in perfect health for a more or less indefinite period, until the introduction of some extraneous factor causes the infection to light up and become active.

This behavior on the part of the parasite he attributes to the possession of a partial and variable degree of immunity on the part of the host. This immunity he believes to be partly natural and partly acquired, the acquired immunity having developed as a result of the prolonged use of quinine as a prophylactic. He believes the disease may become active in patients of this type following upon severe muscular fatigue, wounds, surgical operations and chloroform anæsthesia, overexposure to the sun, or even typhoid inoculations.

Kaminer and Zondek<sup>(17)</sup> report having found parasites in the blood of apparently healthy persons. They say of these persons that at least they were free from fever, and complained of nothing worse than a little headache and a feeling of lassitude.

Of interest in this connection is the observation of Delanoe<sup>(6)</sup> in an epidemic of malaria in the Oulad Hassoun, Western Morocco. A remarkable feature of this outbreak was the early appearance of a large number of gametocytes in the blood of persons stricken with malaria. Similar phenomena have been recorded by other observers, which should serve as an indication that primary malaria must be strictly dealt with, especially in localities where conditions for transmission are favorable.

Malaria as a problem may be regarded from three distinct standpoints: First, there is that aspect of malaria that most frequently comes under the notice of the physician—the active manifestation of the disease, which may express itself in a more or less typical, clinical picture. There are the familiar benign and malignant tertian fevers, with exacerbations occurring about every forty-eight hours; the less frequently observed quartan fevers, with attacks every seventy-two hours; double tertians

and quotidiens, giving rise to the daily attacks of ague; double quartans, mixed tertians and quartans, postponing and anticipating attacks; and the rest of the more or less bewildering series of clinical manifestations to which malaria may give rise, all of which are familiar to practitioners in the tropics. While the experienced man grows to regard with suspicion all pyrexial attacks, and to look for the unexpected picture in malaria, still, it is the part of wisdom to suspend judgment on any atypical case suspected to be malaria until the microscope confirms that suspicion. However, this phase of malaria will not be discussed in the present paper.

Secondly, there is the matter of relapses, which has a very vital bearing on the prevention of malaria among troops. It is impracticable to discuss the theoretical considerations of malarial relapses, for this would involve a discussion of the cytology and physiology of the organism.

Lastly, there is the problem presented by the carriers; that is to say, persons who are parasitized with *Plasmodium*, but show no apparent symptoms of the disease. These constitute the most dangerous group, and the group to which I shall devote the most attention.

The detection of infected, but apparently healthy, recruits and their separation from the healthy and uninfected recruits are matters that will severely tax the knowledge, skill, and resources of any army medical corps; yet this work must be thoroughly done, if the uninfected recruits and the neighboring troops at the front are to be protected against the menace of the carriers.

Once detected, there still remains the task of sterilizing the malarial carrier, and this may prove in some cases a task of almost equal difficulty.

It is not my purpose to outline here a course of procedure that might be followed by army medical authorities, for that is a matter that naturally lies wholly within their province and responsibility. I merely wish to set forth a few of the points of attack of which a protozoölogist might avail himself. These would be entirely aside from clinical data, such as pyrexia, splenic enlargement, and so forth. Under the heading of laboratory procedure we would have to consider:

#### 1. FRESH AND STAINED BLOOD FILMS

The ordinary blood films are not to be relied upon for the detection of carriers. A long search may or may not reveal gametocytes or an occasional trophozoite, suspicious pigmentation of leucocytes or mononucleosis. To state it plainly, the



examination of such preparations with the expectation of discovering even a fair proportion of carriers amounts to a waste of time, pure and simple, unless the general blood picture, in the absence of parasites, yields the evidence of latent malaria.

The Ross thick-film method may be employed. This, in all probability, will yield a higher proportion of positives than the thin smears; still, many will be missed. Reliance must not be placed on this method, if it is desired to secure accurate results.

The differential leucocyte count may, in itself, be sufficient to place a man under suspicion as being a latent malarious subject. Fever and parasites may be absent; but if the blood shows a high percentage of mononuclear leucocytes or if there is occasionally a transient leucocytosis unaccompanied by fever, one may suspect malaria and place the subject under observation.

## 2. CONCENTRATION AND CULTURAL METHODS

There are several of these, all based in principle on the scheme underlying Bass's method of cultivating *Plasmodium*. The original procedure was devised by Bass and Johns<sup>(1)</sup> and involves a careful technic which, however, is not beyond the resources of the advanced student of medicine. Modifications have been devised by Row<sup>(27)</sup> and by J. G. and D. Thomson.<sup>(33)</sup> All of them are practical. In selected cases one may use the technic of Dudgeon and Clark,<sup>(9)</sup> although these authors point out that they have failed to secure results in infections with *Plasmodium vivax* of benign tertian fever. The method of Bass and Johns may probably be considered as the best for precise work.

## 3. PROVOCATIVE METHODS

By these are meant the employment of certain drugs, biological products, or the quartz lamp. Such measures have a tendency to force the parasites out of the spleen, bone marrow, and elsewhere, into the peripheral circulation, where they can be detected on the ordinary blood films and then be dealt with by the regular specific treatment.

James,<sup>(15)</sup> in an able paper, has reviewed the etiology of malarial relapses. Relapses, he says, almost invariably follow the so-called spontaneous cure of primary cases of malaria; that is, the cessation of symptoms without treatment. This is just the class of cases for which we must be very watchful in a body of men drawn from malarious tropical regions, for it is extremely likely that a large proportion of such men never received treatment for the malaria from which they have suffered. Infections insufficiently treated with small doses of quinine will,



James goes on to say, in all probability relapse. If prompt and vigorous treatment is instituted after the onset of an attack of malaria, relapses are less likely to occur; and, conversely, the later the primary attack is treated (even though the doses of quinine given were large and treatment continued for a long time), the more certainly will the symptoms recur.

James says, further, that occasionally relapses will occur when parasites are present in the blood and the patient has not stopped taking quinine. He also believes that the infection will, in time, die out, if reinfection is excluded and death does not take place. This, he adds, applies in the last analysis even to persons who are carriers, but who manifest no febrile symptoms though parasites may be found in the peripheral blood.

Under the first heading, the detection of carriers through the medium of blood films, but little need be added to what has already been said. The difficulties are pretty well understood by all laboratory workers. Macfie and Ingram(18) have called attention to a condition they encountered in West Africa that adds to the difficulty of identifying species of the parasite in blood films. These workers noted the rarity of crescents in the blood of peripheral subtertian infections they encountered in that field. This made necessary the identification of the parasite from the character of the trophozoites, a task that offers some difficulty to the inexperienced microscopist.

Of interest in connection with the examination of blood for malarial parasites is the question as to the number of parasites that are necessary to produce fever. Ross and Thomson(26) have made some studies on sporulating forms in the peripheral blood and give the following figures:

In benign tertian fever 100 adults per cubic millimeter of blood are necessary to produce a fever of 99° F., and 300 adults or more per cubic millimeter are necessary to produce a fever of 100° F., or over.

In malignant tertian fever 3,000 young rings per cubic millimeter of blood are necessary to cause a fever of 99° F., while from 5,000 to 300,000 young rings per cubic millimeter will give a fever ranging from 99° to 106° F.

This has led these authors to state that in a case of true malaria the microscopist is certain to find parasites if the blood is taken at the time of the paroxysm, except in those theoretical cases of malignant tertian fever when all the parasites are sporulating simultaneously. This is in line with the statement attributed to Ronald Ross by Dudgeon and Clark(9) as follows:

But for a broad general rule we may, I think, accept the principle (pending more exact researches) that if we cannot find the parasites after careful search their number is not usually sufficient to produce fever.

This statement of Ross's, made in 1911, with the reserve characteristic of the careful scientist, will certainly not be safe to follow to-day in the handling of carriers, and it is questionable if it is to be strictly followed in the case of active malaria. The theoretical cases mentioned by Ross and Thomson, where malignant parasites mature simultaneously, are sufficiently common occasionally to trouble a microscopist who tries to find the trophozoites of *Plasmodium falciparum* during a febrile attack. In this connection it will be interesting to recall the statements of Garin and of Kaminer and Zondek I have already given. James,<sup>(15)</sup> too, has pointed out the difficulty of attacking the parasites in the parenchyma of the spleen and in the bone marrow with quinine. In this connection he cites cases in which patients dying from malaria showed no parasites in the peripheral blood, but did show a few in splenic smears, where of course the erythrocytes were much less abundant. He also noted that, in cases where the patient died after three to five days' treatment with quinine, the parasites were more frequently seen in the spleen and bone marrow when the quinine had been administered orally, less frequently when it had been administered by hypodermic injection, and least frequently when the quinine had been given in three to four doses of 22.5 grains intravenously. His explanation was that the parasites in the spleen and marrow escape the full effects of the quinine, so that when those in the peripheral circulation are killed some still remain in the spleen and marrow. When these multiply above a certain number, they appear in the peripheral circulation, and further multiplication there brings about a febrile relapse. While much of this does not have a direct bearing on the question of handling carriers, the general point might well be borne in mind.

As to concentration and cultural methods I can do little more than refer the reader to the original papers of the authors to whom I have already alluded. The methods in every case are too detailed and technical to make their presentation here practicable; but many of them are of great and undoubted aid in the detection of parasites in the blood when these are present in numbers too few to admit of discovery on the ordinary blood film except after long and painstaking search.

Some two years ago at a meeting of this society, I ventured to suggest to my medical colleagues the advisability of resorting

to the use of adrenalin for the purpose of dislodging malarial parasites from the internal organs and the fine capillaries. My suggestion passed without comment in the debate. Several other men have since thought of the same thing, have been able to carry it into execution, and have achieved results that, to say the least, are interesting in their bearing on the topic under discussion.

Neuschlosz<sup>(22)</sup> has very recently demonstrated that malarial parasites resting in the spleen parenchyma may be made to enter the peripheral circulation through the exhibition of substances believed by some to have the power of causing contraction of the spleen. It may be said in passing that it is yet to be shown that these drugs produce contraction of the spleen. He has used adrenalin, ergot, and extract of hypophysis. The reaction is accompanied by a typical paroxysm and fever, the parasites appearing in the blood from four to six hours after the injection. According to the observations of Neuschlosz, the parasites make their peripheral appearance more promptly after the administration of adrenalin than they do after extract of hypophysis has been given, but with the latter substance they remain in the circulation for a longer period of time.

Di Pace,<sup>(8)</sup> working on the same principle, has reported success following the administration of salts of berberin and of strychnine—preferably the nitrate.<sup>5</sup>

Mandoki and Maule<sup>(19)</sup> have used Coci's quinine method in the provocation of blood parasitosis in malaria and were able to detect latent malaria in 50 per cent of the cases thus treated. This test offers the objection, however, that it necessitates the exhibition of the quinine for four weeks.

Von Draga,<sup>(35)</sup> in a series of experiments, has succeeded in reviving latent malaria by the injection of sterilized milk. In 5 of his cases there was no reaction; in 13 there was an immediate rise of temperature followed, after an interval of ten to fourteen days, by fever and the appearance of the plasmodia in the blood. In 3 cases he found parasites in the blood, apart from the specific "milk-injection-fever."

<sup>5</sup> The experiments of Di Pace have recently been repeated by King [*Indian Journ. Med. Res.* (1918), 6, 116], who has failed to confirm the results of Di Pace. King states that strychnine, in most cases, does not bring about an increase in the number of parasites in the peripheral circulation. He concludes that the drug offers no aid in the routine diagnosis of latent malaria. As to the action of strychnine on the spleen, King states that in large doses it brings about a reduction in the size of large spleens, but has no effect on spleens that are only slightly enlarged.



Brauer<sup>(2)</sup> employed horse serum in his investigations at Skutari, Albania. In tropical malaria he witnessed an immediate increase in the number of schizonts and gametocytes. Injections of the serum brought out parasites he had been unable to detect in the blood in some cases, and he therefore employed the method in the detection of latent malaria. Supplementary to other treatment he employed injections of horse serum, followed in four hours by an intravenous injection of quinine. His use of milk as employed by von Draga was attended with less success.

Jarno,<sup>(16)</sup> on the other hand, reports having tried Brauer's subcutaneous injections of horse serum in 37 cases of malaria, without success. He says nothing about its applicability to the detection of carriers.

Muller<sup>(21)</sup> adds to these provocative methods the irradiation of the spleen with the quartz lamp.

Reinhard<sup>(25)</sup> holds that in latent malaria quinine fails, because the parasites are not in the general circulation. Therefore, except in relapses, they are inaccessible to quinine treatment unless first carried into the peripheral circulation. As to the cause of relapses, he ascribes them to a purely mechanical factor—the influence of the blood pressure which on elevation would tend to sweep the parasite out of the sinuses and capillaries and into the circulation. This, to say the least, seems quite logical; but at the same time it would seem that other causes as well might bring about the relapses. J. G. Thomson,<sup>(32)</sup> in a careful analysis, has finally disposed of Schaudinn's anomalous theory of the parthenogenesis of the macrogametocyte. It has long been my belief,<sup>(13)</sup> first expressed in 1914, that malarial relapses might be induced by a transient hyperglycemia. Evidence I have since gathered has strengthened that belief and indicates confirmation of the early views of Bass and his coworkers on the influence of blood sugar on the clinical course of malaria. Strikingly suggestive data along these lines has recently been published by de Langen and Schut<sup>6</sup> in their work on tropical acclimatization.

Reinhard quotes Bach as having shown that irradiation of the spleen with the ultra-violet quartz lamp will bring the parasites into the circulation. Quinine is then given. He adds that still better results than those he cites are obtained by the use of the "aureole lamp" of Siemens and Halske.

Finally, in connection with the general problem of examination

<sup>6</sup> *Nederl. Tijdschr. voor Geneesk.* (1918), 1, 336.



of the blood and detection of latent cases, reference should be made to the work of Meyerstein<sup>(20)</sup> in connection with the Wassermann reaction on malarious subjects. He has shown that the Wassermann reaction is frequently positive in tertian malaria during the first days following access of fever. This particularly applies with the ethereal heart extract of Lesser. A positive reaction is seldom obtained after the tenth day, particularly in intractable cases with a tendency to relapse.

Meyerstein holds that the reaction depends on, but does not completely coincide with, the presence of malaria parasites in the blood. He found that the Wassermann reaction disappeared simultaneously with the parasites under the influence of quinine and also in two cases treated with neosalvarsan. The conclusions he draws are: That it is unlikely that the disappearance of the Wassermann reaction during the treatment of a case of malaria signifies a final cure; and that, on the other hand, cases of malaria that—apart from any question of syphilis—give a positive Wassermann reaction must be considered as requiring further treatment.

The outlining of a method of systematic examination of military recruits along the lines laid down in this paper naturally presupposes a comparative study of the work of the several men cited, and the provisional scheme that follows is only put forward as a suggestion of a general method which might, however, be considerably modified with experience. It is taken for granted that all recruits will receive a thorough physical examination and that this, in the natural course of events, would include an inquiry into the previous history of malaria; palpation of the spleen, bearing in mind the tropical splenomegalies of unknown etiology, anæmia, jaundice, cachexia, and the like.

The laboratory examination of the recruits might profitably be taken up in three stages as follows:

#### FIRST STAGE

One Ross film and one or two thin blood smears from each recruit. The thick film is to be used for the detection of the presence of parasites, and the thin films for the identification of species, if the thick film does not yield that information through the presence of crescents; these slides to be taken on two successive days, the morning of the first day, and the late afternoon or evening of the second day if the first specimen is negative. A careful differential leucocyte count should be made

in each case with a view to detecting any mononucleosis. All positives discovered by this test are to be sent to the hospital for treatment; the cases reported negative by the examiners to be passed on to the

#### SECOND STAGE

This involves the utilization of the provocative methods of the first group I have described; namely, adrenalin, hypophysial extract, ergot, strychnine,<sup>7</sup> berberin, quinine.

The choice would naturally depend on a study of the comparative action of the various drugs or upon the individual taste of the military medical officer coöperating in the work.<sup>8</sup>

Positives detected at this stage would be sent to the hospital for treatment, and the negative cases would thereupon pass on to the

#### THIRD STAGE

Here would be used the biological reagents of the second group; namely, milk, horse serum, the quartz lamp.

Positives finally detected here would, of course, go to the hospital for treatment. The negatives, however, should be kept under medical surveillance for a definite period and watched particularly for symptoms following periods of severe physical exertion, muscular fatigue, or exposure.

In this paper it is not my purpose to go into the matter of the treatment of malaria. It seems desirable, however, to call attention to a recent very important contribution to malariology.

Skinner and Carson,<sup>(29)</sup> working in India in 1911, conceived the idea that irradiation of the spleen by the Roentgen rays might bring about the destruction of malarial parasites lying within the substance of that organ. The authors experimented on eleven cases of malaria, some of them very serious, accompanied by splenomegaly, and in some cases running a temperature of more than 104° F. Some of the cases were admitted to the hospital in collapse. These patients were treated by three- to five-minute exposures to the Roentgen rays.

<sup>7</sup> See footnote 5, p. 300.

<sup>8</sup> I have recently undertaken a series of studies on the action of these and other drugs, especially with a view to determining, among other things, their effect on the sugar content of the blood. The work is being undertaken in collaboration with some of my colleagues and will include a clinical and pharmacological as well as a protozoölogical study of the action of the drugs. It is hoped that a preliminary paper may be published in the near future.

In every case splenic pain was relieved, and the engorged spleen reduced. The temperature fell and did not usually rise again; when it did rise, further treatment of the same nature brought it down permanently. Recovery, they reported, was not attended by the anæmia usually present in cases treated with quinine. In no case did the authors fall back upon quinine in cases treated by the Roentgen rays, while they successfully treated cases that had shown no cure on the administration of quinine.

Medical history repeats itself. The work of Skinner and Carson seems to have been practically forgotten for several years, for a search of the literature available to me has resulted in the discovery of no further work along this line until the papers of Pais and of Deutsch published last year, which I have recently seen in abstract.

Pais<sup>(23)</sup> has reported on fifty patients treated by spleen irradiation. His success seems to have been not quite so brilliant as that of Skinner and Carson, but he seems to have been successful in arresting the disease in nearly all cases, and to have brought about a reduction of the spleen to its normal outlines. He states that small doses in a first attack attenuate the disease; larger doses appear to change the cycle of the fever. In his experience new generations of parasites appear to display exalted virulence under the influence of the rays. In connection with chronic cases that have failed to improve under quinine, he states there may be complete recovery under the influence of the Roentgen rays, or at least the infection may be so modified that quinine will work a cure.

Deutsch<sup>(7)</sup> employed deep irradiation of the spleen in 27 cases. Of these 17 were malignant, and the other 10 benign, tertian fever. In almost all there was speedy reduction of the spleen, irrespective of other results. Of the 10 benign tertian cases, 7 were reported as free from fever and gametocytes for a period of six months and were therefore regarded as cured.

The effect of the treatment became apparent, as a rule, after the first application of the rays, when the relapse next due failed to appear. Of the 3 cases that were not benefited, 2 were used in the preliminary experiments and were given treatment with a lower dosage; the other had failed to yield to quinine treatment.

Of the 17 subtertian cases 9 were apparently uninfluenced by the treatment; in 4 others the attacks ceased for a month, and in the other 4 parasites were absent from the blood and there



was no fever over a period of several months. All of these 17 cases had been unsuccessfully treated with quinine and salvarsan.

Just a word in conclusion regarding quinine and the question of quinine-fast parasites. The facts regarding arsenic-fast trypanosomes are fairly familiar, but there is much doubt as to whether or not strains of quinine-fast plasmodia develop following the prolonged use of quinine.

James<sup>(15)</sup> in 1913 stated his belief that the malarial parasite develops some resistance to quinine, and since then several other workers have expressed the same belief.

Teichmann<sup>(30)</sup> has made an interesting series of clinical and experimental studies on quinine habituation and the apparent quinine-fastness of *Plasmodium*. Working in a German military hospital in Turkey, he noted the apparent failure of quinine as a prophylactic for malaria in certain cases of both benign and subtertian fever. He found that patients who had not taken quinine previously responded readily to the regular treatment. On the other hand in those who had taken quinine regularly the parasites appeared as soon as the quinine was withdrawn or even persisted during its administration. He has reviewed the usual explanations for this condition, but notes that many patients were suffering from dysentery and enteritis, so that the quinine was incompletely absorbed. He ascribes much of the trouble to quinine habituation of the body leading to a reduction in, or even disappearance of, the specific action of the drug, and he gives some interesting experimental data in connection with that contention.

He concludes that a certain concentration of quinine in the blood is necessary before the drug will exert its specific action. In quinine habitues smaller and smaller quantities of quinine are there; the quinine-fastness of the parasites is therefore only apparent. The plasmodia are still sensitive to it, but the quinine is not present in sufficient amount to kill them. He therefore gives the quinine intermittently. Teichmann states that he is not opposed to the employment of quinine as a prophylactic; he urges its systematic but careful use. If malaria does develop in spite of the prophylaxis, he advises treatment by the administration of quinine in intermittent and rising doses.

On the other hand, Giemsa and Halberkam,<sup>(11)</sup> reviewing the work along this line, state that they have not been able to confirm the contention of Teichmann, and also that of Neuschlosz, that elimination of the drug is different in those accustomed to quinine than in those who have not been used to taking it.



The results of Russell, (28) who also experimented with intermittent quinine, resemble those of Teichmann. In repudiating the quinine-fast theory, he states his belief that in apparent quinine-fastness of the parasites the trouble is traceable to imperfect absorption of the drug. He remedies this by combining the quinine with iron and strychnine or capsicum and ginger, which combinations in his hands brought about a cessation of the relapses.

But, nevertheless, judging from the behavior of protozoa of this type under adverse environmental conditions, there is biological soundness in the following statement of David Thomson: (31)

Since crescents or gametes (sexual parasites) are the agents which infect mosquitoes, thereby propagating and spreading the disease, it is of extreme importance to rid the blood of them. Small doses of quinine encourage their production and intermittent large doses have the same effect. I hold, therefore, that except in unusual circumstances clinicians who adhere to treatment in small doses, or who give large doses (thirty grains daily) intermittently are doubly guilty of malpractice, and that patients who refuse to undergo a thorough course of treatment are not only foolish to themselves, but are in addition a danger to a tropical community infested with mosquitoes.

To this I may add that our present knowledge regarding gamete carriers should teach us that ridding the blood of gametocytes effectively involves prolonged treatment directed against their progenitors, the trophozoites. I have no knowledge of the length of time a gametocyte may live, as such, in the circulation. The microgametocytes have little to fall back upon during this period in the life cycle of the organism, when food-taking would seem to be impossible, and they probably perish very quickly. The macrogametocytes contain a certain store of food substance which becomes available during sporogony; and, even if this is convertible into energy during life in the blood, there is a limit to it, and once exhausted it would seem that the cell would quickly die. Therefore, the persistence of gametocytes in the blood of a malarial convalescent would appear to be evidence of the presence, somewhere within the host organism, of the asexual forms that are continuously developing into the sexual forms.

Ross's conclusions regarding the efficacy of tatar emetic in the elimination of crescents seem not to be borne out by later work; and, until more proof of its efficiency in this direction is brought forward, we must, in the work of sterilizing carriers,

continue to repose faith in active treatment directed against the trophozoites.

#### SUMMARY

1. The recruiting of large bodies of men destined to form army units, from areas in tropical countries where malaria is known to be endemic or epidemic, is certain to bring together many men who, apparently healthy, are yet carriers of the malarial parasite and are capable of conveying it to healthy persons. It likewise imposes a heavy responsibility upon the medical staff of any military organization so obtaining its recruits.

2. These carriers, in the presence of anopheline mosquitoes, are a source of peril to any community that is comparatively free from the malarial fevers. They are likewise a peril in their own garrison, and great care should be exercised in the selection of a site on which to establish a training camp.

3. Such men, on undergoing the heavy work of military training, with its attendant fatigue and exposure, are extremely likely to develop the disease in its active form with the consequence that the effective strength of their unit will be reduced.

4. At the front, in a foreign country where antimosquito measures may be impracticable and quinine prophylaxis fraught with difficulties, these carriers are a source of peril to all neighboring troops, especially as they may be the bearers to the troops of other nations of new and virulent strains of the parasites.

5. Carriers should be sought out with care. No reliance should be placed on the simple examination of blood films; some will be detected by this method, but many will escape detection. Use should be made of concentration or cultural methods, supplemented in the negative cases by provocative methods that will tend to awaken the latent infections and bring the parasites into the peripheral circulation, where they can be destroyed by the usual specific treatment.

6. In the event of quinine failing to act, investigation should be made to see if the drug is being absorbed and turned into the blood stream in sufficient volume to bring about the destruction of the organism. If the drug is not being utilized by the patient, either the condition should be corrected by the use of adjuvants or some other form of treatment should be instituted.

7. If all of these measures fail, the recruit should be honorably discharged from the service.

8. The surgical importance of malaria in connection with wounds and operative procedure should likewise be well borne in mind by the surgical staff. The tendency of latent malaria to become active under the influence of wounds, surgical procedure, and anaesthesia is well established by several observers.

## REFERENCES

- (1) BASS, C. C., and JOHNS, F. M. A method of concentrating malaria plasmodia for diagnostic and other purposes. *Amer. Journ. Trop. Dis. & Prev. Med.* (1915), 3, 298.
- (2) BRAUER, GREGOR. Ueber Mobilisierung von Malaria-Parasiten im Blute. *Wien. klin. Woch.* (1917), 30, 105.
- (3) Burma. Report on Sanitary Administration of Burma, for 1916 (1917), Rangoon. Office Supt. Govt. Printing, p. 11.
- (4) CARLES, J. Treatment of malaria. *Journ. de Med. de Bordeaux* (1918), 89, 3.
- (5) CREMONESE, G. The 1917 epidemic of malaria. *Gazz. degli Ospedali e delle Clin. Milan* (1918), 39, 153.
- (6) DELANOE, P. Contribution a l'etude du paludisme au Maroc Occidental. I. L'Epidemic palustre des Oulad Hassoun. *Bull. Soc. path. exot.* (1917), 10, 586.
- (7) DEUTSCH, FELIX. Tiefelstrahlung der Milz bei Malaria. *Wien. klin. Woch.* (1917), 30, 207.
- (8) DI PACE, I. Per la diagnosi della malaria latente. Morgagni. (1915), 57, Pt. 1, No. 7, 266.
- (9) DUDGEON, L. S., and CLARK, CECIL. On the cultivation of the malarial parasite in vitro. *Journ. Trop. Med. & Hyg.* (1917), 20, 104.
- (10) GARIN, CH. Sur la genese du Paludisme. *Presse Med.* (1917), 25, 319.
- (11) GIEMSA, G., and HALBERKAM, J. Quinine in the human organism. *Deut. med. Woch.*, Nov. 29, 1917. Reviewed in *Med. Rec.* (1918), 93, 345.
- (12) GILL, CLIFFORD A. The prevention of malaria in war with special reference to the Indian Army. *Journ. Roy. Army Med. Corps.* (1917), 29, 439.
- (13) HAUGHWOUT, F. G. The tissue invasive powers of the flagellated and ciliated protozoa with especial reference to *Trichomonas intestinalis*. A critical review. *Phil. Journ. Sci., Sec. B* (1918), 13, 217.
- (14) HOFFMAN, FREDERICK L. Malaria as a factor in military efficiency. *Southern Med. Journ.* (1917), 10, 676.
- (15) JAMES, S. P. The etiology of relapse in malarial fever. Cited by Thomson, No. 31.
- (16) JARNO, LEO. Ueber Mononucleose bei Malaria. *Wien. klin. Woch.* (1917), 30, 914.
- (17) KAMINER and ZONDEK. *Deut. med. Woch.* Cited in *Med. Rec.* (1917), 92, 1073.
- (18) MACFIE, J. W. S., and INGRAM, A. Observations on malaria in the Gold Coast Colony, West Africa. *Ann. Trop. Med. & Parasitol.* (1917), 11, 1.



- (19) MANDOKI, L. and MAULE, W. The effects of neosalvarsan in malaria. *Munch. med. Woch.*, Feb. 12, 1918.
- (20) MEYERSTEIN. Die Wassermannsche Reaktion bei Malaria. *Munch. med. Woch.* (1917), 64, 366.
- (21) MULLER, L. R. The treatment of malaria. *Munch. med. Woch.*, Jan. 29, 1918.
- (22) NEUSCHLOSZ, S. The experimental forcing out of the malarial parasites into the circulation. *Munch. med. Woch.*, Jan. 22, 1918.
- (23) PAIS, A. Influence of Roentgen rays on malaria. *Gazz. degli Ospedali e delle Clin. Milan.* (1917), 38, 1121.
- (24) Philippine Islands. Quarterly Rept. Health Serv. 3d quarter (1917) Manila, Bureau of Printing (1917), p. 51.
- (25) REINHARD, P. Ueber Provocation latenter Malaria durch Bestrahlung mit ultravioletten Licht. *Munch. med. Woch.* (1917), 64, 1193.
- (26) ROSS and THOMSON. Cited by Thomson, No. 31.
- (27) ROW, R. On a simplified technique of Bass's method of cultivating malarial parasites in vitro and a few observations on the malarial parasites cultured by this technique. *Indian Journ. Med. Res.* (1917), 4, 388.
- (28) RUSSELL, FREDERICK F. Relapses in malaria. *Proc. Med. Assoc., Isthmian Canal Zone* (1916), 9, Pt. 1, 25.
- (29) SKINNER, BRUCE, and CARSON, H. W. Curative influence of Roentgen rays in malaria. *Brit. Med. Journ.* (1917), No. 2617, 431.
- (30) TEICHMANN, FRIEDRICH. Klinische und experimentelle Studien über die Chiningewohnung des menschlichen Körpers und die scheinbare Chininfestigkeit der Malaria Plasmodien. *Deut. med. Woch.* (1917), 43, 1092.
- (31) THOMSON, DAVID. The diagnosis and treatment of malarial fever. *Journ. Roy. Army Med. Corps* (1917), 28, 658.
- (32) THOMSON, J. G. Notes on malaria. *Journ. Roy. Army Med. Corps* (1917), 29, 379.
- (33) THOMSON, J. G., and D. The cultivation of one generation of benign tertian malarial parasites (*Plasmodium vivax*) in vitro by Bass's method. *Ann. Trop. Med. & Parasitol.* (1913), 7, 153. The growth and sporulation of the benign and malignant tertian malarial parasites in the culture tube and in the human host. *Ibid.*, 509.
- (34) VANDENBOSCHE. *Journ. de med. et de chir. prat.*, April 25, 1917. Cited in *Brit. Med. Journ.*, No. 2956 (1917), p. 7.
- (35) VON DRAGA, LUDWIG T. E. Die experimentelle Aktivierung latenter Malariafälle. *Wien. klin. Woch.* (1917), 30, 102.
- (36) WOODCOCK, H. M. Protozoological experiences during the summer and autumn of 1916. *Journ. Roy. Army Med. Corps* (1917), 29, 290.



# A STUDY OF THE CALCIUM GLANDS IN THE COMMON PHILIPPINE HOUSE LIZARD<sup>1</sup>

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## TWO PLATES

A calcium deposit in the region of the neck posterior to the opening of the external auditory meatus was noted in many snakes, frogs, and lizards and described by Wiedersheim(3) many years ago. He says in part:

Auf deren Nervenendstelle ruht eine dünne Membrana tectoria von Hufeisenform, und darüber findet sich eine Kleine Aussammlung von Otolithen.

Der bei Fischen und Amphibien auf manchen Excursionen angetroffene Ductus Endolymphaticus hat jene auch bei den Reptilien noch nicht eingestellt und das bei vielen bis unter das Schädeldach reichende Ende stellt bei Embryonen von Eidechsen und auch von Schlangen bei Kalkkrystalle führendes Säckchen vor, welches, weis durch die Haut schimmernd, mit blosem Auge erkannt wird. Bei Ascalaboten geht von daher eine weitere Entfaltung aus. Das Säckchen tritt an der Parieto-occipitalnaht durch diese um sich subcutan zwischen der Musculatur des Nakkens, zum Theil auch des Schultergürtels, als vielfach gebuchteter Schlauch zu vertheilen, bis zum Pharynx und der ventralen Seite der Halswirbelsäule herab. Eine weiche Otolithenmasse erfüllt ihn.

The functional activity of this gland seems, however, to have been entirely neglected, and no observations have been recorded. Boulenger(1) noticed this gland, but believed, with Sauvage, that it was a pathological condition. He states:

From New Caledonia I have examined one specimen, presented by M. Delacour to the Paris Museum, and described by Dr. Sauvage as *Lepidodactylus crepuscularis*, Bavay. One of the characters pointed out by Dr. Sauvage as distinguishing the supposed latter species from *L. lugubris*, viz. the presence of a large gland on each side of the neck, is an individual (apparently pathological) character, and occurs in many species of the Geckonidæ.

Stejneger(2) also describes this calcium deposit on the lateral side of the neck as follows:

On each side of the neck behind the ear opening there may be seen in many geckos a more or less enlarged oblong swelling which, in large

<sup>1</sup> Received for publication May 2, 1918.



specimens, often assumes great dimensions—equalling one-half the size of the skull. This sac is filled with calcareous matter, which is connected with the ear by ducts or canals. Professor Wiedersheim, who was the first to describe this organ in detail, considers it an auxiliary to the auditory apparatus, having as its object the perfecting of the sense of hearing in the animals. These calcareous masses are popularly, but erroneously, believed to be the undeveloped eggs which the females are supposed to carry about in a sort of pouch until they are deposited in some safe place for hatching.

While severing the spinal cord in the region of the medulla, I was attracted by a grayish white substance that oozed from the region of the neck. This substance soon became hard on exposure to the air. This gland containing the milky substance was constant in two species of the pregnant house lizards. Under the microscope this milky substance was seen to be of an amorphous nature when fresh, but on exposure to the air small crystals were formed. In all probability these were crystals of calcium carbonate. At first it was believed that this calcium gland was present only in pregnant lizards and that it probably bore a definite relation to the formation of the calcium shell about the egg substance. However, the gland is present both in the male and in the female, but there is an increased functional activity during pregnancy.

I am indebted to Mr. E. H. Taylor, of the Bureau of Science, for the proper classification of the Philippine house lizards, for much material given me, and for many specimens that were loaned to me, for all of which I wish to express my appreciation.

There are four common Philippine house lizards found in Manila: *Cosmybotus platyurus*, *Hemidactylus frenatus*, *Peropus mutilatus*, and *Hemidactylus luzonensis*. The calcium gland is found in only two of the above-named species, *Cosmybotus platyurus* and *Peropus mutilatus*. In *Hemidactylus frenatus* and *H. luzonensis* no calcium is stored in the gland in the region of the neck. However, in these two species a spongy reticulum is found in the region of the neck, which will be described more in detail in a subsequent paragraph. All four species are ovoviviparous, the embryo developing to a length of more than 1 millimeter before the egg is laid.

The calcium glands are found, one on each side of the neck, lying immediately behind the opening of the external auditory meatus. They are slightly oblong, the greatest diameter being in an anteroposterior direction. Their position is semicircular, the extremities reaching the anterior and posterior sides of the neck (Plate II, fig. 1). The gland in the pregnant *Cosmybotus platyurus* is much the largest, having an average anteroposterior

diameter of 8.3 millimeters on the right side, while the gland on the left side has an average length of 7.3 millimeters. The average lateral diameter was 6.3 millimeters. The calcium glands of pregnant individuals of *Peropus mutilatus* are somewhat smaller, having an anteroposterior diameter of 5.4 millimeters and a lateral diameter of 4.2 millimeters. In all pregnant lizards where the egg substance was almost ready to receive its calcium shell the gland was always found engorged with calcium milk. In several instances after the shell had been completely formed and the eggs were ready to be laid the calcium gland was found to be practically devoid of its calcium content. When the gland is engorged with calcium milk, small, filled canaliculi are seen to communicate with the occipitoparietal sinus, which also is filled with this milky fluid. The females in which the eggs were not developed showed the same type of gland as found in the male. All male lizards of this species that were examined contained only a comparatively small amount of calcium in the gland, while in a number there was no calcium deposit whatever. In no instance was the gland found enlarged and engorged as it was in the pregnant lizards. The average anteroposterior diameter of the calcium gland in the male specimens was 4 millimeters, while the lateral diameter was 3 millimeters. It is plainly evident that the functional activity of the calcium gland is greatly increased during the period when the calcium is secreted to form the shell about the egg substances.

In *Peropus mutilatus* the gland is somewhat smaller than in *Cosmybotus platyurus*. However, the same general conditions obtain in this species as in *Cosmybotus*. The gland in the pregnant lizard is always large and is filled with amorphous calcium. In the male the gland, in a large proportion of specimens, contained no calcium deposit whatever.

In both species the gland, when engorged, can be easily seen lying underneath the skin. In *Cosmybotus platyurus* the gland lies somewhat more superficially than in *Peropus mutilatus*. When an effort is made to remove the skin of *Cosmybotus*, it sometimes adheres firmly to the gland, so that the latter is broken. In *Peropus* the gland lies more deeply—well below the superficial fascia, and therefore the overlying skin and the gland never adhere to each other. In both species the gland is well surrounded by voluntary muscle fibers.

The calcium glands were also studied in a large series of the gecko (*Gekko gekko*), which is a species of lizard very much larger than the house lizard. In all the females, many of which were pregnant, a calcium deposit was present in the gland. In

twenty of the male specimens studied a calcium deposit was found in only four, while in the remaining number there was a complete absence of the calcium. Here, again, is the striking difference in the functional activity of the gland in the pregnant, the nonpregnant, and the male lizards.

Taylor, in an unpublished observation, has also seen a calcium deposit in many of the young lizards after hatching.

The calcium gland in *Cosmybotus platyurus* and *Peropus mutilatus* are histologically identical. Under low-magnifying power the gland is distinctly lobulated, with a pattern much like the external surface of a mammalian lung. There is no distinct capsule, and the external wall is surrounded by a loose connective tissue reticulum. From the external wall trabeculae extend into the gland, dividing it into follicles. Each follicle is lined with a single layer of large cuboidal epithelial cells that lie on a basement membrane. The connective tissue intervening between follicles is extremely loose in texture. Between the adjacent walls of the follicles is a rich capillary network. In the active gland the follicle is filled with an amorphous substance into which the cells have wandered. The cells that lie near the epithelial wall retain their cellular characteristics distinctly. As they wander farther out into the center of the follicle, they become more and more disintegrated. Whether or not these cells take an active part in the production of the amorphous calcium is not definitely known, although it seems probable that they do play some rôle in the formation of calcium by the gland. The peripheral follicles are, as a rule, smaller than those placed more centrally. Voluntary, or striped, muscles often extend with the trabeculae into the medullary portion of the gland.

In *Hemidactylus frenatus* and *H. luzonensis* the gland is composed of a reticular structure forming numerous small follicles, which have never been found to contain a deposit of calcium. However, in the pregnant lizards a calcium milk is found filling the sinuses on the superior and medial sides of the orbital cavity as well as the sinus in the region of the occipitoparietal suture.

#### SUMMARY AND CONCLUSIONS

The calcium gland in the region of the neck of the common house lizard is undoubtedly an auxiliary to the auditory organ, inasmuch as it supplies a calcium salt for the formation of the otoliths of the ear.

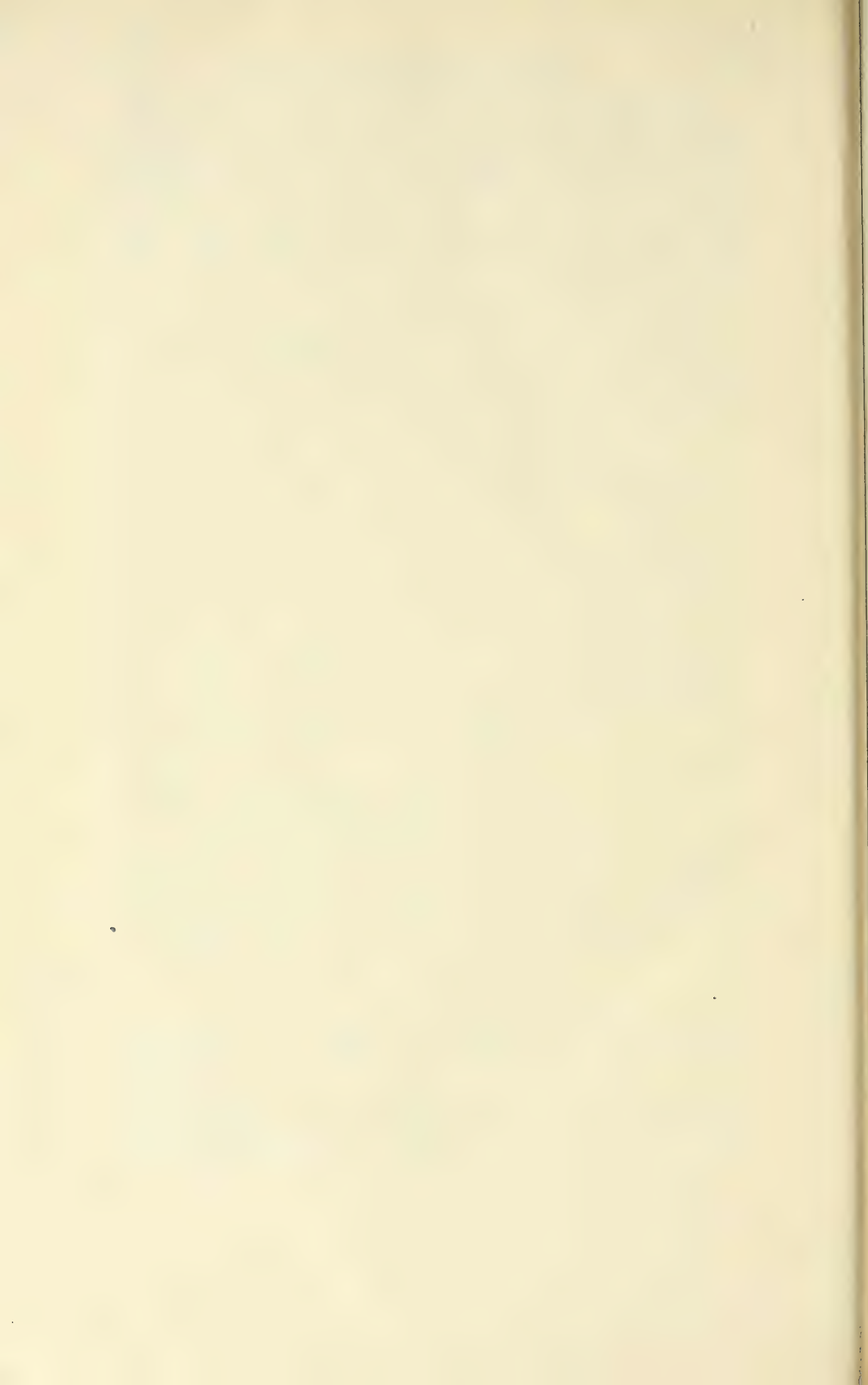
In pregnant lizards the calcium gland is thrown into a greater functional activity, which immediately decreases when the calcium shell about the egg substance is formed.



According to Wiedersheim the calcium gland is an auxiliary to the auditory apparatus. On the one hand, it may be possible that this gland, during pregnancy, becomes more active, thus increasing the keenness of the auditory sense rendered necessary at that time for the protection of the parent and her progeny. On the other hand, there is evidence to show that the calcium gland prepares the calcium salt that is carried by the blood stream to the oviduct, where it is deposited as the shell surrounding the egg substance.

## REFERENCES

1. BOULENGER, G. A. *Proc. Zool. Soc. London* (1883), 121.
2. STEJNEGER, LEONHARD. *Proc. U. S. Nat. Mus.* (1899), 21, 787.
3. WIEDERSHEIM, GEGENBAUER. *Verg. Anat.* (1898), 1, 890.



## ILLUSTRATIONS

### PLATE I

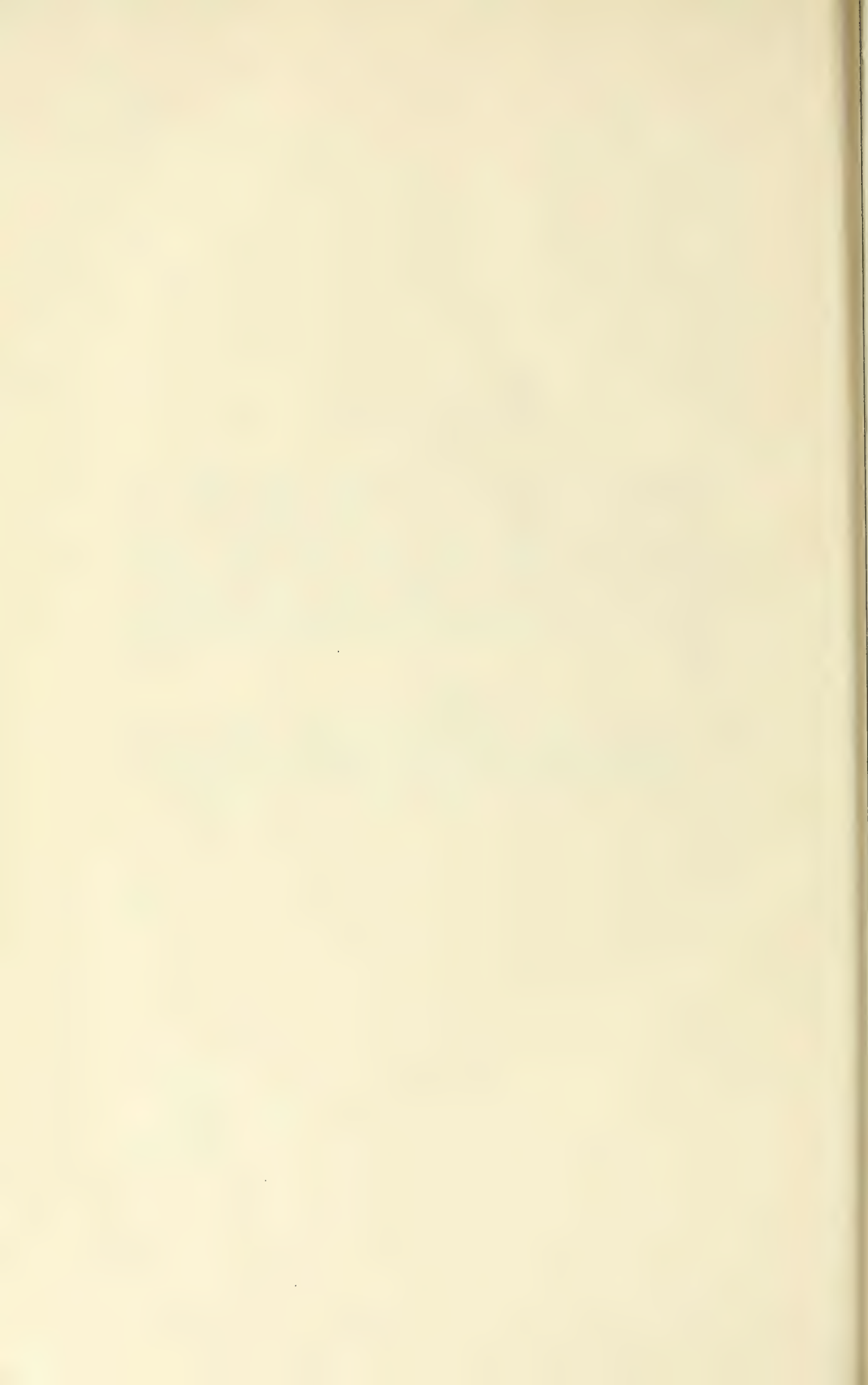
From left to right, lizards 1 and 2, *Hemidactylus frenatus*, male and female, showing a complete absence of calcium deposit in the gland. In No. 2, the calcium shell completely covers the egg substance. Lizards 3 and 4, dorsal view, are *Cosmybotus platyurus*, pregnant females, showing large engorged calcium gland on each side of the neck. The last two illustrations show ventral views of lizards 3 and 4, illustrating the extent of the calcium gland on the ventral side of the neck. The eggs are almost fully developed in 3 and 4, and are ready to receive the surrounding deposit of calcium to form the shell.

### PLATE II

[Drawings by V. de los Santos.]

- FIG. 1. Low-power drawing of calcium gland, showing follicles containing an amorphous calcium deposit.  $\times 140$ .  
2. High-power drawing, showing cells wandering into the amorphous calcium deposit in the follicle.  $\times 670$ .





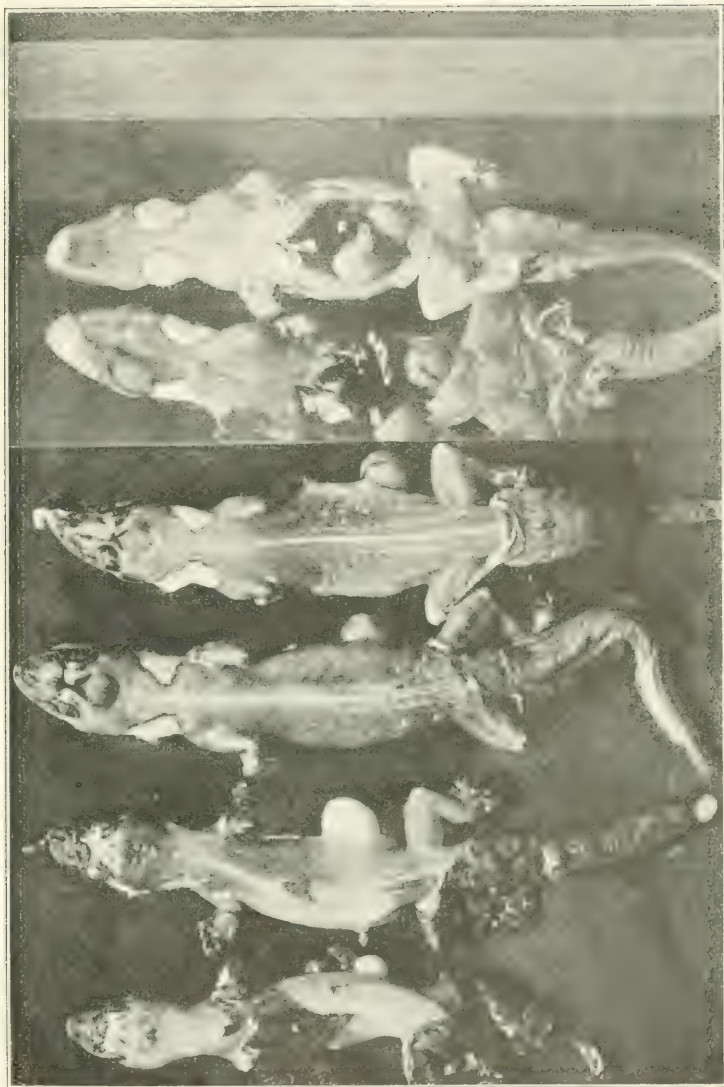


PLATE I.



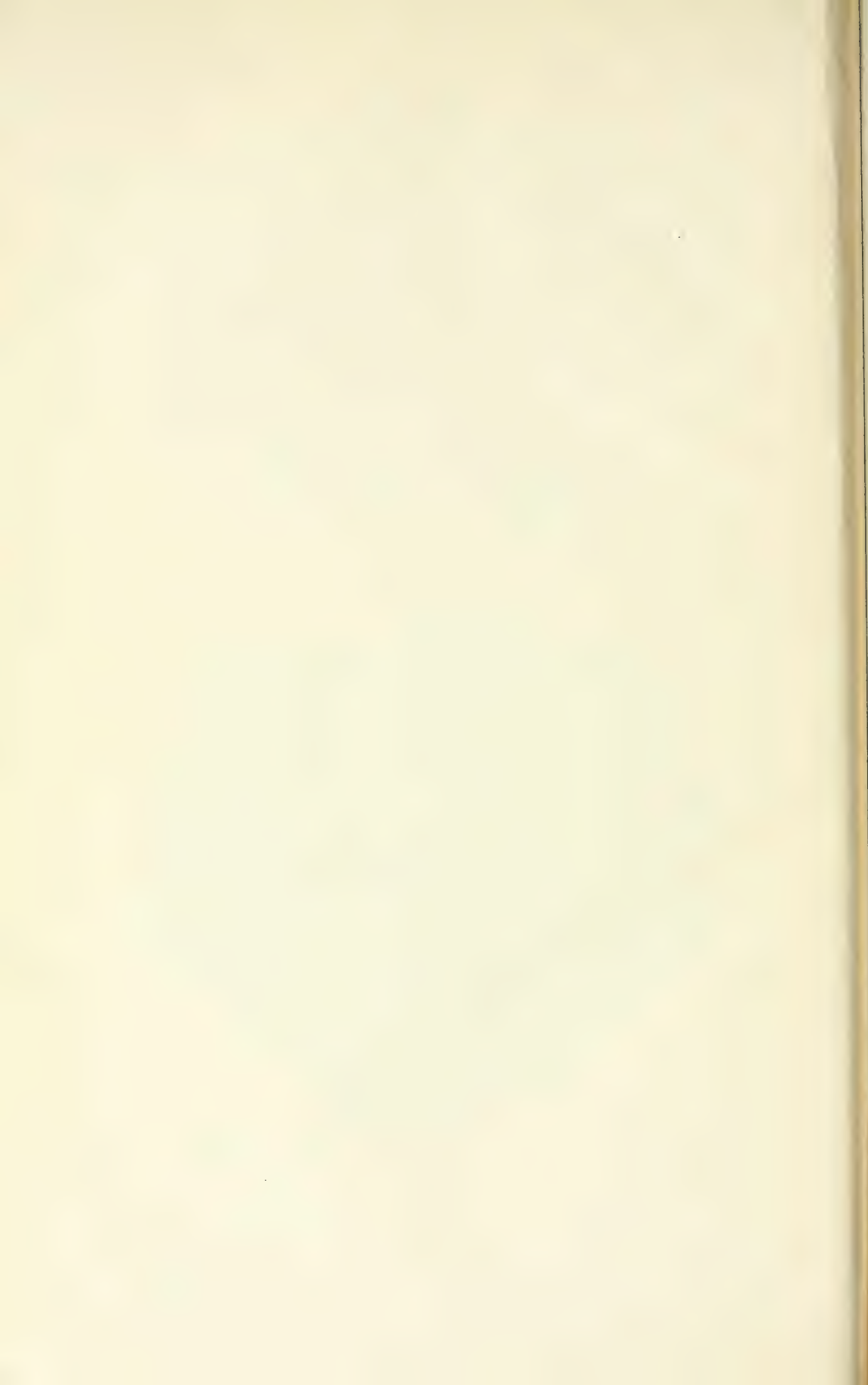




FIG. 1. Low-power drawing of calcium and gland, showing follicles containing an amorphous calcium deposit.  $\times 140$ .



FIG. 2. High-power drawing, showing cells wandering into the amorphous calcium deposit in the follicle.  $\times 670$ .



# A STUDY OF ONE HUNDRED THIRTY-FIVE HUMAN EMBRYOS AND FŒTUSES COLLECTED IN THE PHILIPPINE ISLANDS <sup>1</sup>

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The human embryos figured in this publication were all secured in the Philippine Islands, most of them from Manila and a few from the surrounding provinces. Several of the specimens are of European parentage and are so indicated; all others are Filipinos, belonging to the Malayan race. Beyer(2) divides the Filipino people (exclusive of the Negritos) into four fundamental types: Short and tall Mongol, Primitive Caucasian, and Indonesian. It will be of interest to see whether a numerical index can be prepared to determine these types in the early stages of development.

The splendid embryological collection that is rapidly growing has been made possible only by the coöperation that is being received from the physicians and surgeons of the Philippine Islands. I take this opportunity to thank all the physicians who have generously donated material to promote the science of embryology in the Philippine Islands. In the period of three years almost one hundred fifty specimens have been collected. Much is yet to be accomplished in the field of embryology, and it is hoped that many more of the earlier stages of the developing human embryo may be secured.

The measurements of all specimens were taken immediately after reaching our laboratory. The embryos are, as a rule, sent to us in a fixing fluid, the larger ones in 10 per cent formalin, the younger ones in Bouin's fluid.

The crown-rump and standing-height measurements are taken according to Mall's method. All the smaller specimens are weighed after being in the preserving fluid for some time, while some of the larger fœtuses are weighed in the fresh state.

The menstrual age was obtained only in a limited number of cases, and no definite conclusions can be drawn at this time as to the size of the embryo for a definite age. However, there seems to be a parallelism between the length of the Filipino

<sup>1</sup> Received for publication May 9, 1918.



and European embryos for a given age in the several cases for which data are at hand. Much more information must be accumulated before any definite statement along this line is justified. Albert and Arvisu<sup>(1)</sup> have taken a large series of measurements on Filipino children from 6 to 36 months of age. A comparison of the height of Filipino and a series of American children measured and reported by Grover<sup>(4)</sup> is almost identical up to the tenth month. In fact, a comparison of the two tables shows that during the seventh or eighth month of life the Filipino children have a greater length than American children. The weight of Filipino children is invariably greater than in American children of the corresponding age after the sixth month. After the tenth month the difference in size between American and Filipino children at once becomes apparent. Whether this difference is due to an environmental cause or a hereditary influence has not yet been determined. Undoubtedly heredity plays a very important rôle, but other causes and influences should not be overlooked. Gibson and Concepcion,<sup>(3)</sup> in a series of five puppies fed on cows' milk, found that growth stopped in four of the animals when 44 days old. Following the administration of an efficient vermifuge there resulted the passage of many ascarids. Growth was immediately reëstablished. The growth charts of these dogs are published in connection with another problem. This points out the importance of considering influences that undoubtedly have a bearing on the growth rate of an individual. That the Filipino is shorter in stature is a well-known fact, but the explanation for this has not yet been satisfactorily determined.

It will be necessary to study a large series of specimens in order more thoroughly to understand the causes that bring about miscarriages, abortions, and premature births. Mall<sup>(5)</sup> was the first to show this importance, and his contribution marks the beginning of investigations in this new field. Experimental teratology in the last two decades has done much toward explaining the formation of many of the pathological specimens and monstrosities. As the physicochemical laws of life are studied more and more, the laws governing the formation and development of monstrosities will be made more comprehensive. The production of cyclopes by the experimental work of Stockard<sup>(8)</sup> and of Werber<sup>(9)</sup> is one of the first decisive steps along this line. Our conception of the formation of certain types of monsters, especially the cyclopes, has been made clearer by the work of Stockard. He found that by treating fish embryos with a mixture of sea water and magnesium chloride over 50 per cent

of the fertilized ova would develop into cyclopes, or one-eyed monsters. Werber obtained similar results by treating fish eggs with very dilute solutions containing acetone and butiric acid.

Table I shows a complete list of the embryos collected in the Philippine Islands. All measurements are computed in millimeters. The crown-rump measurement is taken with calipers from the vertex of the head to the most distal region of the buttocks. The standing height, according to Mall,<sup>(6)</sup> is the crown-rump plus the thigh and leg length. The thigh length is taken by describing an arc about the head of the femur, representing the distance from the head of the femur to the tip of the buttocks. The distance from the described arc on the thigh to the knee joint represents the length of the thigh below the buttocks. This is necessary, as otherwise the distance from the head of the femur to the described arc, which represents the distance from the head of the femur to the distal tip of the buttocks, would be duplicated, if the embryo is measured when body and lower extremities are placed in a straight line.

The measurements of the chorionic sac were taken in three dimensions. The weight of the embryos is taken in grams. Only a few of the smaller ones were weighed in the fresh state; all others were taken after having been in a fixing fluid for some time. In the terminology of the pathological specimens an attempt is made to use terms that indicate to which group the embryo belongs as adopted by Mall,<sup>(7)</sup> who divided his pathological specimens into seven groups. This is especially adaptable for the first four groups, in which no embryo is present. A brief history of the mother is given, her age, number of pregnancies, full-term pregnancies, and abortions. The menstrual age is reckoned from the first day of the last menstrual period to the date of abortion.

As stated in Table I, out of 266 pregnancies, 183, or 64.4 per cent, were full-term births; 75, or 28.6 per cent, abortions; and 8, or 3.0 per cent, ectopic. The percentage of abortions in my series is a great deal higher than Mall reports, who found approximately 80 full-term births to every 20 abortions. In this series there are approximately 70 full-term births to every 30 abortions. The percentage here will be probably reduced when a larger series of cases is collected. The causes of abortion here are probably due in a large proportion of cases to general systemic diseases, such as beriberi, and other tropical diseases. Certainly a comparatively few, if any, are self-induced. Venereal disease is also almost unknown here, especially among the laboring class. There is, however, a great deal of endometritis

TABLE I.—Showing complete list of human embryos collected in the Philippine Islands.

Catalogue No.	Crown-rump dimension.	Standing height dimension.	Chorionic sac dimension.	Weight of embryo.	Sex.	Normal or pathological specimen.	Nationality.	Age of mother.	Number of pregnancy.	Abortion.	Ectopic pregnancy.	Menstrual age.	Type of pregnancy.	Physician donating specimen.
	mm.	mm.	mm.	gms.								Days.		
117			14 × 12 × 7			Chorionic sac.	F						Uterine	E. Perez.
115			17 × 16 × 7			Chorion and amnion	F	37	12	7	5	38	do	Mañalang.
48			18 × 15 × 10			do	F						do	R. Molina.
135			30 × 18 × 22			Umbilical stump	F						do	R. Lopez.
121			35 × 25			Chorionic villi	F	27	3	2	0	1	Right tubal	P. Guazon.
134			40 × 22 × 18			Nodule	F	24	1	0	1	65	Uterine	B. Valdez.
23			49 × 37 × 25			Two amniotic sacs and chorion.	F	25	7	5	2	99	do	B. C. Crowell.
133			50 × 35 × 20			Chorion sac.	F					? 90	do	A. Gutierrez.
129			53 × 38 × 20			Vesicle of cord	F					? 90	do	J. E. Reed.
123			63 × 46 × 22			Chorion and amnion	F					? 90	do	De los Angeles.
27			71 × 54 × 25			do	F	25	2	1	1	79	do	V. Reyes.
124						Hydatidiform mole	F	20	2	0	2	78	do	V. Magno.
131						Chorion and amnion	F					? 90	do	J. E. Reed.
132							F						do	A. Gutierrez.
122						Chorionic villi and sac	F	28					Left tubal	P. Guazon.
125						Scattered chorionic villi	F	35	7	5	1	1	do	Do.
126						Chorionic villi	F	45	5	4	0	1	Right tubal	Do.
127						Chorionic villi and sac	F	21	2	1	0	1	Left tubal	G. Santos.
128						Degenerated chorionic villi.	F	32					Right tubal	P. Guazon.
130						Vesicular nodule.	F	30	8	6	1	1	Tubal	Reyes.
96	1.43		31 × 18.5 × 8			Cyst of umbilical cord	F	25					Uterine	De Leon.
32	1.5		25 × 22 × 14			Anencephalia	F	28	8	7	1		do	V. Reyes.
8	2.6		33 × 22.5				F	28	7	5	2		do	E. Perez.
	2.6		45 × 33.5 × 22				F	35					Tubal	Vincent.



20	3.6	13×10.5×7.5	Normal	F	26				Uterine.	Manalang.
14	3.65	14×12×8	do	F	26				Tubal.	A. Reyes
15	4.13	68×23	Spina bifida	F	36	1	0	1	Uterine.	Santos.
21	6.5		Anencephalia	F	19	5	3	1	Tubal.	Calderon.
33	6.5		Cystic tail	F	25				do	Gilman.
1	8	65×40×25	Atrophic	F	28	6	5	1	Uterine	Guazon.
5	8.5	85×63×35	do						do	Reed.
52	10		Normal	E	24	4	2	2	do	Castañeda
95	10	32×22×15	Anencephalia	F	37	1	0	1	do	Nolasco.
111	10	12.5×13×8	Back of embryo attached to amniotic sac.	F					do	Guazon.
41	14		Normal	F	22				Tubal.	Gonzales.
43	15		do	F					Uterine	Guerrero.
40	15?		Macerated	F					do	Yabini.
18	16	51×43×25	Anencephalia	F	42	11	7	4	do	Garcia.
13	16	47×35×15	Atrophic head	F					do	Velarde.
45	17.2		Normal	F					Tubal	Gilman.
18	17.5	28×22×16	do	F					do	Nolasco.
29	18.5	40×30×15	do	E	35	3	2	1	Uterine	Manlove.
12	21	61×48×15	Fetus compressus	F					do	Reed.
19	24	30	Normal	F	25				do	Guazon.
36	24.7	27.7	do	F	36	5	4	1	do	Perez.
39	26	30	Hydrocephalus	F	30	3	2	1	do	Guazon.
97	29	36	Normal	F					do	Lemmon.
42	32.3	41.5	Exomphaly	E	33	4	1	2	Right tubal.	
01	32.3	60×30×17	Partial cyclopia	F	20	2	1	1	Uterine.	
44	40.5	52	Club hands and feet.	F					do	
83	40.6	54	Normal, cleared	F					do	
16	43.5	56	Amniotic bands attached to fingers.	F	39	5	4	0	Right tubal.	
94	45.5	60	Normal	F	29	8	4	4	Uterine.	Ocampo.
98	47	66.5	do	F					do	Perez.
59	51	6.90	Club hands and feet	F					do	

\* F. Filipino.

Е, European.

TABLE I.—Showing complete list of human embryos collected in the Philippine Islands—Continued.

Catalogue No.	Crown-rump height dimension.	Chorionic sac dimension.	Weight of embryo.	Sex.	Normal or pathological specimen.	Nationality.	Age of mother.	Number of pregnancy.	Full term.	Abortion.	Ectopic pregnancy.	Menstrual age.	Type of pregnancy.	Physician donating specimen.
	mm.	mm.	gms.									Days.		
4	53	75	8	♀	Normal.	F	24	7	3	4			Uterine	Calderon.
46	53	80		♀	do	F							do	H. Sison.
84	54	79			Normal, cleared								do	
26	55	72	7.3	♀	Normal.								do	
58	55	79	4.60		Club hands and feet.								do	
30	59	76	8.2	♂	Hernia of liver	F	27	4	3	1		98	do	Angeles.
51	66	98	20	♂	Club extremities macerated.	F							do	Calderon.
2	66	93	29	♂	Normal.	F	27	5	4	1			do	Guazon.
74	67	100	15.2	♀	do	F	28	10	9	1		? 97	do	Calderon.
9	68	99	27.8	♂	do	F							do	
116	73	99	13.81	♀	Slight clubbing of extremities.	F							do	Cabarrus.
25	73	103	24	♂	Normal.								do	
65	79	113	37	♂	Amniotic band constricting neck.								do	
78	87	126	50	♂	Normal.								do	Perez.
99	87.5	124	28.7	♂	do	F							do	Smith.
17	88	128	38.4	♀	do	E							do	Acosta Sison.
49	90	129	45	♂	do	F	19	3	2	1		73	do	Perez.
100	92	136	49	♂	do	F							do	Lemmon.
105	95	144	45.5	♀	Umbilical hernia	F	28	3	2	1			do	Calderon.
7	96	145	63.5	♀	Normal.	F	39	10	9	1			do	Albert.
28	103	147	63.5	♂	Normal, cleared	F							do	

108	103	151	54	Sex	Amniotic bands attached to extremities; twin to 109.	F	27	1	0	1	do	Calderon.
24	104.5	151	64	♀	Normal.	F						
85	105	155		♂	Normal, cleared	F					do	Angeles.
57	111	165	95	♂	Normal.	F					do	
109	112	163	84	♀	Amniotic bands attached to extremities; twin to 108.	F	27	1	0	1	do	Calderon.
72	120	186	112	♀	Normal, macerated	F					do	Do.
86	120	190		♂	Normal, cleared	F					do	Do.
90	125	193.5	119	♀	Normal.	F	27	5	4	1	158	Castañeda.
110	135	196.5	110	♂	Amniotic band constricting left ankle.	F					do	Do.
87	135	200		♀	Normal, cleared	F					do	Do.
75		c 32 > 43		♀	Normal.	F					do	
55	137	199	205	♀	do	F	33	3	2	1	do	Parrish.
47	141	214	240	♀	do	E	26	3	1	2	do	Sately.
91	144	220.5	192	♂	do	F					do	Castañeda.
88	148	210		♂	do	F					do	
50	149	224	295	♀	do	F	35				do	Calderon.
89	150	220		♂	do	F					do	
104	150.8	233		♂	Normal, cleared	F					do	
67	151.5	231.5	129	♂	Normal, macerated	F					do	
53	152	232	227	♂	Normal.	F	22	5	3	2	do	
70	158	246	412	♂	do	F	39	9	8	1	do	Calderon.
102	160	255	339	♂	Normal; twin to 103	F	21	2	1	1	do	Lemmon.
22	162.5	234.5	224	♂	Normal.	F	24	5	4	1	do	Feliciano.
64	164	252	350	♂	do	F					do	
56	164	260	375	♀	do	F					do	Castañeda.
60	167	255	364	♂	do	F	30	1	0	1	123	Calderon.
103	171	265	357	♂	Normal; twin to 102	F					do	
71	171	272	355	♂	Normal.	F					do	

c Lower extremities only.

TABLE I.—Showing complete list of human embryos collected in the Philippine Islands—Continued.

Catalogue No.	Crown-rump dimension.	Standing height-dimension.	Chorionic sac dimension.	Weight of embryo.	Sex.	Normal or pathological specimen.	Nationality.	Age of mother.	Number of pregnancy.	Full term.	Abortion.	Ectopic pregnancy.	Type of pregnancy.	Physician donating specimen.
	mm.	mm.	mm.	gms.										
93	174	264		567	♀	Cyst on left side of head; oedematous.	F	22	4	3	1	Days. 7 174	Uterine	Calderon.
77	178	297		555	♂	Normal	F						do	
107	179	260		370	♂	do	F						do	Perez.
61	179	281		555	♂	do	F	22	3	2	1		do	Calderon.
69	180	291		500	♂	do	F						do	
80	183	281		459	♀	do	F						do	
73	184	331		862	♀	Hernia of mid brain; cleft palate; hare lip.	F						do	Parrish.
76	185	269		367	♂	Normal	F						do	
37	186	276	147×90×12	497	♀	do	F	26	6	4	2		do	Parrish.
79	186	288		452	♀	do	F						do	
63	186	293		420	♂	do	F	27	7	6	1		do	Bautista.
66	192.5	303		537	♀	do	F						do	
62	195	287		870	♀	Oedematous; stunted extremities.	F	20	3	2	1		do	Ferrer.
81	195	320.5		610	♀	Normal	F						do	
54	198	303	155×115×50	975	♂	do	F	18	1	0	1		do	Parrish.
82	212	342.5		905	♂	Hernia of liver	F	38	8	7	1		do	Do.
120	224	338		712	♂	Normal	F						do	
92	224	367		1,074	♂	do	F						do	
38	230	299		1,340	♀	Oedematous; round head	F	28	11	10	1		do	Parrish.
31	230	320		1,440	♂	Ectrodactyia anencephalia.	F	17	1	0	1	240	do	Cabarrus.
14	231	359		829	♂	Normal	F	18	3	1	2		do	Pond.
12	233	363		1,210	♂	do	F	22	3	1	2		do	Do.



10	248	384	1,432	♂	Normal	F	36	8	7	1	?	198	do	Perez.
34	252	397	1,762	♂	Hydrocephalus	F	23	3	2	1			do	Parrish.
13	273	424	2,037	♂	Normal	F	23	3	2	1			do	Tolentino.
11	273	429.5	1,800	♀	Edematous condition of right eye.	F							do	Norris.
35	273	440	2,110	♀	Anencephalia	F	20	2	2	0			do	Parrish.
119	280	451	1,935	♂	Hernia of liver	F							do	Do.
106	280	460	1,034	♂	Split upper eyelids.	F								
3	330	510	2,110	♂	Normal	F	17	1	1	0				Pond.
68	337	467	2,160	♂	Edematous; stunted extremities.	F								Parrish.
Total.								266	133	75	8			

among the common people, especially in multipara, in which the uterine passages are not properly treated following parturition. This undoubtedly accounts, in a degree at least, for a larger percentage of abortions.

TABLE II.—*Showing the number of normal and pathological specimens from uterine and ectopic pregnancies.*

Month.	Growth.	Uterine specimens.		Ectopic specimens.		Total.
		Nor-mal.	Patho-logical.	Nor-mal.	Patho-logical.	
	<i>mm.</i>					
January	0- 2.5	0	5	0	1	6
February	26- 25	7	20	3	9	39
March	26- 68	11	7	0	2	20
April	69-121	13	5	0	0	18
May	122-167	18	1	0	0	19
June	168-210	14	3	0	0	17
July	211-245	4	3	0	0	7
August	246-284	2	5	0	0	7
September	285-316	0	0	0	0	0
October	317-336	1	1	0	0	2
Total		70	50	3	12	135

Total number of uterine specimens, 120, or 88.8 per cent.

Total number of ectopic specimens, 15, or 11.2 per cent.

Table II shows the number of normal and pathological specimens obtained both from uterine and ectopic pregnancies. The numerical index indicating the rate of growth per month is taken from the one adopted from Mall. The largest number of abortions occurred in the second month of gestation. The number then gradually decreases in each succeeding month, with only two abortions in the last month of pregnancy. The largest percentage of pathological specimens, 120, or 88.8 per cent, are uterine, and 15, or 11.2 per cent, are ectopic pregnancies. The total number of normal specimens is 73, or 54.8 per cent, while the pathological specimens number 62, or 46 per cent. Of this latter number 37 per cent are from uterine and 8.8 per cent are from ectopic pregnancies.

#### REFERENCES

- (1) ALBERT, J., and ARVISO, T. Physico-mental development of Filipino children. Read at the III Asamblea Regional de Medicos y Farmacéuticos de Filipinas, February 11 (1916).
- (2) BEYER, H. O. Unpublished classification of basic types of Filipino people.

- (3) GIBSON, R. B., and CONCEPCION, I. The influence of fresh and autoclaved cows' milk on the development of neuritis in animals. *Phil. Journ. Sci., Sec. B* (1916), 11, 119.
- (4) GROVER, J. I. Some measurements of normal children especially of the leg and arm. Some interesting deductions and practicable possibilities. *Arch. Pediat.* (1915), 32, 473.
- (5) KEIBEL and MALL. Human Embryology. J. B. Lippincott Company, Philadelphia and London (1910), 1, 202.
- (6) IDEM. *Ibid.* (1912), 2, 180.
- (7) MALL, F. P. On the frequency of localized anomalies in human embryos and infants at birth. *Am. Journ. Anat.* (1917), 22, 49.
- (8) STOCKARD, C. R. The development of artificially produced cyclopean fish—the "magnesium embryo." *Journ. Exp. Zool.* (1909), 6, 285–338.
- (9) WERBER, E. I. Experimental studies aiming at the control of defective and monstrous development. A survey of recorded monstrosities with special attention to ophthalmic defects. *Anat. Rec.* (1915), 9, 529.





## REVIEWS

**The Elements of the Science of Nutrition**, by **Graham Lusk**, Ph. D., Sc. D., F. R. S. (Edin.) | professor of physiology at the Cornell University Medical College, | New York City | third edition, reset | Philadelphia and London | W. B. Saunders Company | 1917 | Cloth, pp. 1-641.

This third and enlarged edition of Professor Lusk's book needs no recommendation to those who are acquainted with his former valuable presentations of this subject. To those who have not had access to Professor Lusk's book it may be said that it is a thorough and scholarly presentation of the general methods, data, and facts of our present day knowledge of nutrition in health and disease, to which he personally has contributed so much.

R. B. GIBSON.

**A Laboratory Manual and Text-Book of Embryology**, by **Charles William Prentiss**, A. M., Ph. D., late professor of microscopic anatomy, Northwestern University Medical School, Chicago | revised and extensively rewritten by **Leslie Brainerd Arey**, Ph. D. | associate professor of anatomy, in the Northwestern University Medical School, Chicago | second edition enlarged | with 388 illustrations | many in color | Philadelphia and London | W. B. Saunders Company | 1917 | Cloth, pp. i-viii+1-411.

The second edition of Prentiss' Text-Book of Embryology as revised by Professor Arey, is indeed improved and brought more up to date. While preserving the general arrangement of the book, the text has been made more readable and clearer, especially to the student, by important additions and some excellent new illustrations. The rearrangement of some of the very long paragraphs is also of decided advantage. The new chapter on the morphogenesis of the skeleton is a valuable and needed addition. It is unfortunate that this edition also failed to include a bibliography, though this is to a certain extent compensated by the more frequent mention of names of authors and investigators. This book like its predecessor should prove of great help both to teachers and students of embryology.

A. GARCIA.

**Chemical Pathology** | Being a Discussion of General Pathology from the Standpoint of | the Chemical Processes involved | By | **H. Gideon**

Wells, Ph. D., M. D. | professor of pathology in the University of Chicago and in | Rush Medical College, Chicago; director of the | Otho S. A. Sprague Memorial Institute | third edition, revised and reset | Philadelphia and London | W. B. Saunders Company | 1918 | pp. 1-707. Cloth, \$4.25, net.

#### From the preface to the third edition:

Despite the war, active investigations in the chemical problems of disease have continued, even in those countries most deeply involved in the conflict. Although some of the later publications of foreign countries have not been directly accessible, but few have not been available at least through abstracts, and it is believed that most of the literature of importance, within the scope of this book, has been considered in its revision, although the rule previously followed of quoting only from the original articles has of necessity been violated in several instances. The new additions to our knowledge in the three years since the second edition was issued have been so numerous that it has again been necessary to reprint the entire work. Several subjects have been largely rewritten, especially Gout, Specificity of Immunological Reactions, Anaphylaxis, Icterus, Acidosis, Diabetes and Uremia. New sections have been added on the Abderhalden Reaction, Specificity, Chemical Basis of Growth, Atrophy, and the Pressor Bases, as well as many briefer additions.

The Principles | of | Hygiene | A Practical Manual for Students, | Physicians, and Health-officers | By | D. H. Bergery, A. M., M. D., Dr. P. H. | Assistant Professor of Hygiene and Bacteriology, University of | Pennsylvania | Sixth edition, thoroughly revised | Philadelphia and London | W. B. Saunders Company | 1918 | pp. 1-543. Cloth, \$3.50 net.

#### From the preface:

This book has been prepared to meet the needs of students of medicine in the acquirement of a knowledge of those principles on which modern hygienic practices are based; to aid students in architecture in comprehending the sanitary requirements in ventilation, heating, water-supply, and sewage-disposal; and to aid physicians and health officers in familiarizing themselves with the advances made in hygienic practices in recent years.

The rapid strides made in our knowledge of the entire subject of hygiene has rendered such a book, based upon the more recent discoveries, almost a necessity to students of medicine.

No attempt has been made to treat the subject in an exhaustive manner, the object being merely to give the general principles upon which the health officer and the physician work in their respective capacities in dealing with conditions which are detrimental to health or which tend to improve health.

The entire range of subjects comprising the comprehensive field of hygiene has not been discussed, but all those subjects which appeared to the author to be most important for those for whom the book has been prepared have received the consideration which their relative importance demanded.

The metric system of weights and measures has been employed throughout the work except in quotations, because this system is now in general

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use in all scientific laboratories in the United States, and because it is in every way preferable to the cumbersome and complicated system, with its various units, which is still in common use.

Handbook | of | Suggestive Therapeutics | Applied Hypnotism | Psychic Science  
| A manual of practical psychotherapy, designed | especially for the  
practitioner of medicine, surgery, and dentistry | By | Henry S.  
Munro, M. D. | Omaha, Nebraska | Fourth edition, revised and en-  
larged | St. Louis | C. V. Mosby Company | 1917 | Cloth, pp. 1-481.

The Venereal Diseases | A Manual of Treatment | An Outline of their  
Management, Prepared | for the Use of Medical Officers of the Army  
| from the office of the Surgeon General of the Army | September  
15, 1917 | Chicago | American Medical Association, 535 North Dear-  
born Street | 1917 | Cloth, pp. 1-100.

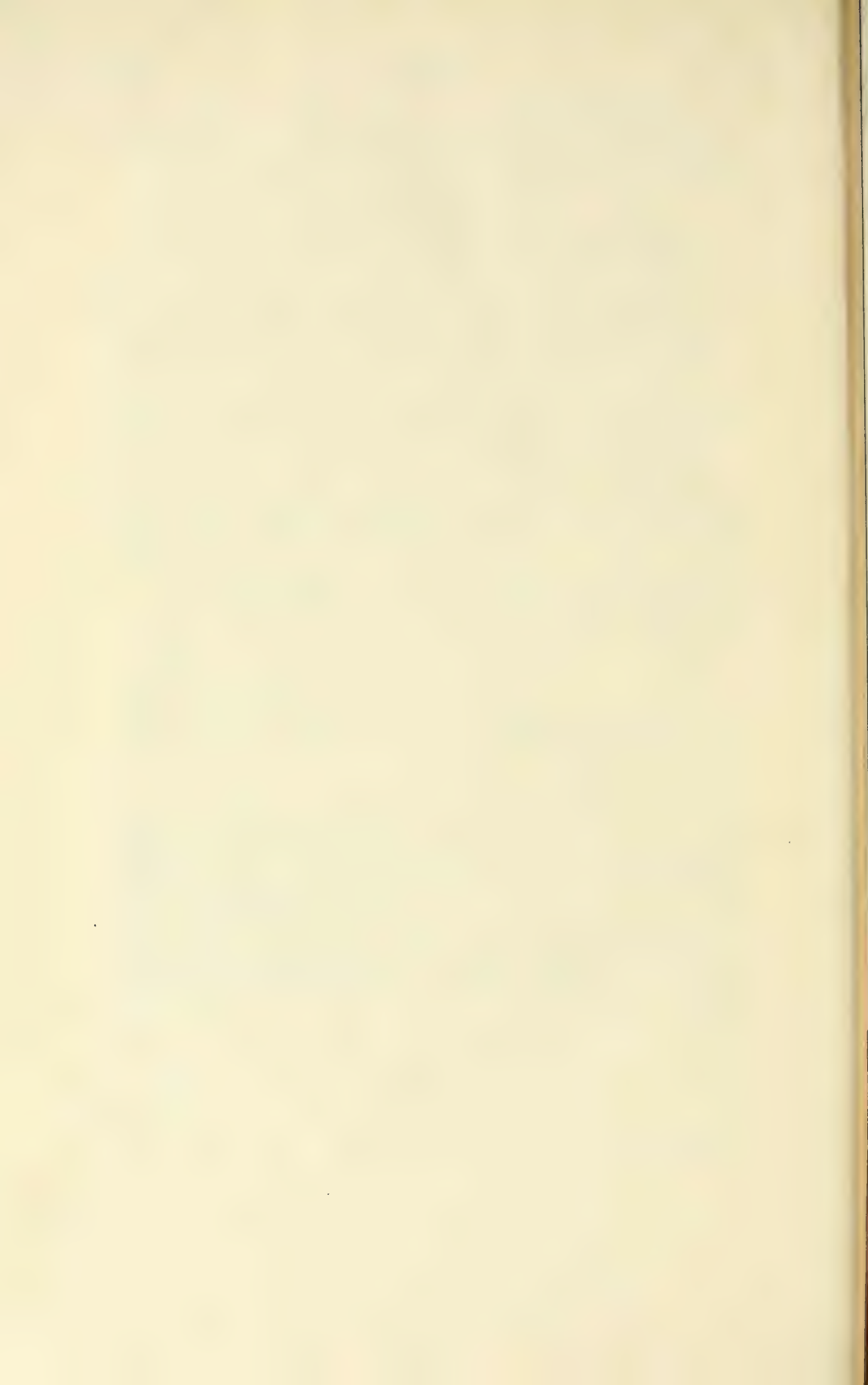
Collected Papers | of | The Mayo Clinic | Rochester, Minnesota | edited by  
| Mrs. M. H. Mellish | Volume IX | 1917 | Philadelphia and London  
| W. B. Saunders Company | 1918 | Cloth, pp. i-ix+1-866, inclusive  
of index.

This volume consists of some hundred or more papers, most of them illustrated, on a variety of subjects of interest to the practitioner. The contributors, thirty-five in number, are all members of either the Mayo Clinic or the Mayo Foundation for Medical Education and Research, and some of both.

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#### From the preface:

It is a patriotic impulse that impels the author, a neophyte, to make this effort to explain, as lucidly as he may, this appliance and its proper application. He realizes that it could be taught much better by master to pupil; having the knowledge and art passed on from one to another. It is his hope that this may be done, until the great usefulness and efficiency of this splint becomes known and thoroughly popularized. This, then, is the object of this little book. It is written for men thoroughly acquainted with anatomy and the subject of fractures in general. No effort is made to compile a "big book." It is the desire to be as concise as possible, and with singleness of purpose teach the virtues of the Hodgen extension suspension splint.





## ERRATA

Page 258: In line 10, *for* THOMPSON *read* THOMSON.

Page 261: In line 27, *for* Bahr *read* Sandwith.

Page 261: In line 28, *for* (1) *read* (6).



## PROCEEDINGS OF THE MANILA MEDICAL SOCIETY

REGULAR MONTHLY MEETING, AUGUST 5, 1918

### MINUTES OF THE MANILA MEDICAL SOCIETY

The regular meeting of the Manila Medical Society was held at the College of Medicine and Surgery, August 5, 1918, at 8.45 in the evening, with Dr. F. W. Vincent in the chair.

The following members were present:

Dr. F. W. Vincent.  
Dr. J. Albert.  
Dr. R. B. Gibson.  
Dr. H. W. Wade.  
Dr. I. Concepcion.

Dr. F. Calderon  
Dr. S. de los Angeles.  
Prof. F. G. Haughwout.  
Dr. R. Fernandez.  
Dr. H. Velarde.

Dr. D. de la Paz.

The minutes of the previous meeting were read and approved.

The recommendation of the council with regard to the applications of Drs. Miguela G. Baltazar, Lamberto Leiva, and Florencio Lara for active membership was ratified by the society.

The chairman announced the resignations of Drs. B. C. Crowell and H. G. Maul as councillor and vice-president of the society, respectively. Their resignations were accepted by the society with regret. The chairman also announced the absence for the remainder of the year of Dr. E. S. Ruth, a councillor for three years. He appointed Doctors Albert, Gibson, and Concepcion to constitute a committee on the nomination for vice-president and two councillors.

The society took a short recess.

At the second session the nomination committee submitted the following nominations:

Major John H. H. Scudder, for vice-president.  
Dr. H. W. Wade, for councillor to succeed Doctor Crowell.  
Dr. P. Guazon, for councillor *vice* Doctor Ruth during his absence.

These nominees were unanimously elected.

On motion of Professor Haughwout, duly seconded, the following resolution was unanimously approved:

That a committee of three be appointed to draft an appreciation of the late Dr. Paul Clements, the same to be spread upon the minutes of the

Manila Medical Society and the Philippine Islands Medical Association and a copy thereof to be transmitted to the family of Dr. Clements.

That this committee include at least one man closely associated with Dr. Clements.

In accordance with this resolution the president appointed a committee consisting of Professor Haughwout, chairman, and Doctors Long and de los Angeles.

Dr. de los Angeles then read a very interesting paper on The Medical Aspect of Criminology: Its Bearing on the Philippines. At the conclusion of the reading of this paper the president asked to be excused on account of an urgent call. Doctor Gibson was then requested to preside over the meeting in the absence of the vice-president elect, Doctor Scudder.

With Doctor Gibson in the chair, the discussion of Doctor de los Angeles' paper was opened, Doctors Gibson, Calderon, Leiva, and Wade, Professor Haughwout, and the author participating in the discussion.

Dr. Lamberto Leiva followed with a paper entitled Mosquitoes around Manila and Vicinity—a Health Problem, which was discussed by Doctors Gibson and Wade, and Professor Haughwout.

The last paper, which was on Endemic Malaria in the Philippine Islands as a Military Problem, was read by Professor Haughwout. This paper was discussed by Doctors Gibson, Wade, and de la Paz, Professor Haughwout closing the discussion.

The meeting adjourned at 11.05 in the evening.

D. DE LA PAZ,  
*Secretary-Treasurer.*

#### SCIENTIFIC PROGRAM

#### THE MEDICAL ASPECT OF CRIMINOLOGY: ITS BEARING ON THE PHILIPPINES

By DR. SIXTO DE LOS ANGELES

A plan of procedure is presented for the purpose of establishing a necessary and fundamental system of study and classification of criminals in the Philippine Islands by taking into account the influence of the natural factors of crime generally accepted by criminal anthropologists and sociologists. The report includes a study of the cephalic indices by regions between Filipino criminals and noncriminals, and the incidence of the various cranial anomalies among forty-four dead Filipino criminals



in relation to the character of the crimes for which they were convicted.

#### MOSQUITOES AROUND MANILA AND VICINITY: A HEALTH PROBLEM

By DR. LAMBERTO LEIVA

Attention was called to the presence of both the culicine and anopheline mosquitoes around Manila and vicinity. Their relation to disease was discussed briefly. The suggestion also was made that the new and shortened route between the yellow fever zone and Manila brought about by the opening of the Panama Canal may allow certain yellow fever cases which are as yet in their incubation period to pass our quarantine stations unnoticed. The danger, therefore, lies in the possibility of the presence of a Philippine species of mosquito able to transmit yellow fever. So far, *Stegomyia fasciata persistans* Banks has not been proved to be a carrier of this disease. This mosquito and other species of the genus *Stegomyia* are indigenous to the Philippine Islands.

It was stated that two important factors are at work against the spread of malaria in Manila: First, nearly every malaria patient receives ready and prompt treatment with quinine, thereby leading to a great reduction of gametocyte carriers; secondly, the systematic work of the "mosquito brigade," for which no inconsiderable credit is due.

Mosquito control was next discussed. A few remarks were made on the study of the life history of the mosquito as being of importance in applying methods of mosquito extermination. The organization and workings of the "mosquito brigade" of Manila were briefly described. The difficult situation that arises is that a few men are not able to inspect and oil breeding places as frequently as is necessary; namely, every twelve days. It was pointed out that systematic work of this nature is necessary to prevent a new generation of mosquitoes being given a lease of life after the preceding brood has been exterminated.

Mosquitoes, if let alone, are always a menace to the safety and comfort of the human population. It is a health problem that calls for active measures of a sanitary campaign—a condition where eternal vigilance is truly the price of safety, and a false sense of security is fraught with grave danger to even this community.—L. L.

ENDEMIC MALARIA IN THE PHILIPPINE ISLANDS  
AS A MILITARY PROBLEM

By Professor F. G. HAUGHWOUT

Professor Haughwout's paper dealt mainly with the problem of latent malaria and "malarial carriers" as likely to manifest themselves in the assembling of large bodies of men recruited from endemic centers of malaria. He outlined and discussed some of the methods that have been employed in the detection of latent malaria. The paper also discussed the matter of "quinine-fast" parasites, and recent work on the treatment of malaria by the use of the Roentgen rays.—F. G. H.

R. B. GIBSON,

*Editor of the Proceedings,  
Manila Medical Society.*

## PROCEEDINGS OF THE MANILA MEDICAL SOCIETY

REGULAR MONTHLY MEETING, SEPTEMBER 2, 1918.

### MINUTES OF THE MANILA MEDICAL SOCIETY

The meeting was called to order at 8.40 in the evening at the Philippine General Hospital by the president, Dr. F. W. Vincent.

Twelve members were present and one visitor, Major Frank Suggs, of the Medical Corps, United States Army, and medical staff of the Philippine General Hospital.

The minutes of the previous meeting were read and approved.

President Vincent presented the applications of Drs. Amparo Concha, Joaquina E. Tirona, Facundo Esquivel, and A. L. Lejano for active membership. It was moved and seconded that the secretary cast the ballots in favor of the applicants. Carried.

Dr. Potenciano Guázon, author of the first paper of the evening, was absent.

Dr. Gervasio Santos presented a case report of A Vesical Calculus of Unusual Size.

Dr. Regino G. Padua read a paper on Cystolithiasis among the Filipinos in Association with Dietetic Deficiency, which was discussed by Doctors Gibson, Wade, and de Leon.

The meeting was adjourned at 10.15 in the evening.

D. DE LA PAZ,  
*Secretary-Treasurer.*

### SCIENTIFIC PROGRAM

#### A CASE REPORT OF A VESICAL CALCULUS OF UNUSUAL SIZE

By DR. GERVASIO SANTOS

A report of a case of vesical calculus was presented, and the stone, which weighed over 700 grams, was exhibited. The composition of the calculus had not been determined. The large size of the stone and its evident rapid rate of formation were the unusual characteristics in this case.

#### CYSTOLITHIASIS AMONG THE FILIPINOS IN ASSOCIATION WITH DIETETIC DEFICIENCY

By DR. REGINO PADUA

The observations of Osborne and Mendel on rats indicate a possible association of dietetic deficiency with the formation of

phosphatic urinary calculi. The Filipino diet is essentially of an insufficient and limited character, particularly from its avitaino nature. The results of the present investigation show that a relation apparently exists between the general dietetic inadequacy and deficiency among the Filipinos and the incidence of phosphatic calculi, in contrast with the reported predominance of uric acid and urate calculi in Europe and the United States.

R. B. GIBSON,

*Editor of the Proceedings,  
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